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Contents

A Reflection Spectrophotometric Study of Ultraviolet Erythema and Pigmentation. M. T. JANSEN 1053
Inorganic Phosphate Response to Intravenous Glucose Administration in Progressive Muscular Dystrophy. D. W. VAN BEKKUM AND A. QUERIDO 1061
Two Cases of Postural Hypotension Showing a Deficiency in Release of Nor-Epinephrine and Epinephrine. ROLF LUFT AND ULF S. VON EULER 1065
Filter Paper Electrophoresis of Human Hemoglobin. DANIEL L. LARSON AND HELEN M. RANNEY 1070
Concentration Gradients for Inorganic I^{131} and Chloride in Mixed Human Saliva. NORBERT FREINKEL AND SIDNEY H. INGBAR 1077
The Demonstration of Type Specific Streptococcal Antibody by a Hemagglutination Technique Employing Tannic Acid. FLOYD W. DENNY, JR. AND LEWIS THOMAS 1085
Pulmonary Function Studies in Mitral Stenosis before and after Commissurotomy. M. HENRY WILLIAMS, JR. 1094
Oxygen Tension of Tissues by the Polarographic Method. VI. Effect of Changes in Position on Oxygen Tension of the Skin of Toes. PHYLLIS G. WILLIAMS, HUGH MONTGOMERY, AND ORVILLE HORWITZ ... 1097
The Effects of Venous Congestion of the Limbs upon Renal Clearances and the Excretion of Water and Salt. I. Studies in Normal Subjects and in Hypertensive Patients before and after Splanchnicectomy. ROBERT W. WILKINS, CLARENCE M. TINSLEY, JAMES W. CULBERTSON, BELTON A. BURROWS, WALTER E. JUDSON, AND CHARLES H. BURNETT 1101
The Effects of Antimicrobial Agents upon Choline Degradation in the Intestinal Tract. J. DE LA HUERGA, PAUL GYÖRGY, SHELDON WALDSTEIN, RICARDO KATZ, AND HANS POPPER 1117
Effect of Phenylbutazone (3,5 Dioxo-1,2-Diphenyl-4-n-Butylpyrazolidine) on Renal Clearance of Urate and Other Discrete Renal Functions in Gouty Subjects. TSAI FAN YÜ, JONAS H. SIROTA, AND ALEXANDER B. GUTMAN 1121
Hexamethonium in the Chronic Treatment of Hypertension—Its Effect on Renal Hemodynamics and on the Excretion of Water and Electrolytes. RALPH V. FORD, JOHN H. MOYER, AND CHARLES L. SPURR. 1133
An Intracardiac Catheter with Inflatable Balloon for Producing Increased Venous Pressures in the Venae Cavae of Man. SAUL J. FARBER AND LUDWIG W. EICHNA 1140
Electrolyte and Water Excretions and Renal Hemodynamics during Induced Congestion of the Superior and Inferior Vena Cava of Man. SAUL J. FARBER, WILLIAM H. BECKER, AND LUDWIG W. EICHNA .. 1145
The Effect of Application of Tourniquets to the Legs on Cardiac Output and Renal Function in Normal Human Subjects. FRANCIS W. FITZHUGH, JR., ROBERT L. MCWHORTER, JR., E. HARVEY ESTES, JR., JAMES V. WARREN, AND ARTHUR J. MERRILL 1163
The Influence of Glucose, Fructose, and Insulin on the Metabolism of Leukocytes of Healthy and Diabetic Subjects. SAMUEL P. MARTIN, GORDON R. MCKINNEY, R. GREEN, AND CAROLINE BECKER 1171
The Combined Effect of Cortisone Acetate and Desoxycorticosterone Acetate in Adrenalectomized Rats on a Low Sodium Diet. ABBIE I. KNOWLTON, EMILY N. LOEB, HERBERT C. STOERK, AND WILLIAM C. MOHLER 1175
A Comparison of Renal Blood Flow Results Obtained in the Intact Animal by the Nitrous Oxide (Derived Fick) Method and by the Para-Amino-Hippurate (Direct Fick) Method. HADLEY L. CONN, JR., JOHN C. WOOD, AND CARL F. SCHMIDT 1180
The Competition between P-Amino-Hippuric Acid (PAH) and Diodrast for Renal Excretion and Extraction in Man. BERTIL JOSEPHSON, JAN EK, HÄRJE BUCHT, LARS WERKÖ, JULIA KALLAS, AND ARNE GRIEG 1184
The Effect of Mechanical Exsufflation on Respiratory Gas Exchange in Chronic Pulmonary Emphysema. R. M. CHERNIACK 1192

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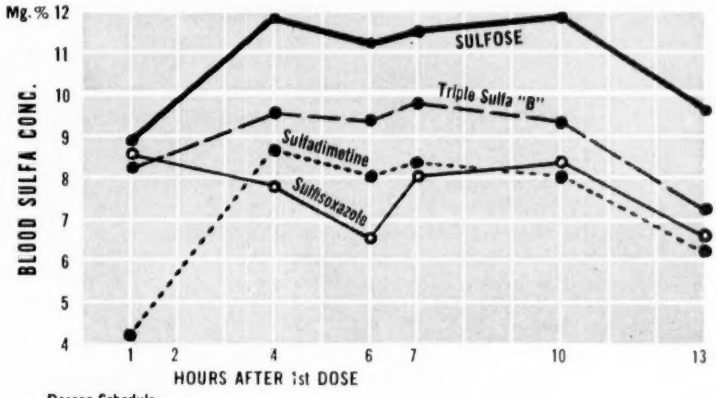

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2. Berkowitz, D.: Antibiotics & Chemotherapy 3:618 (June) 1953
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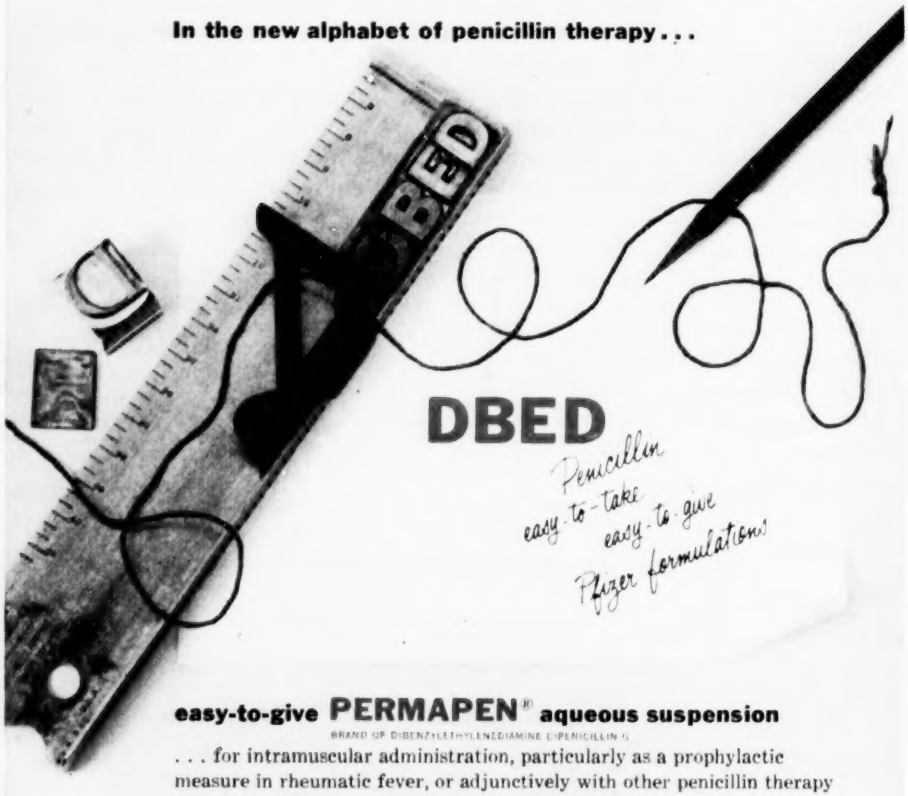

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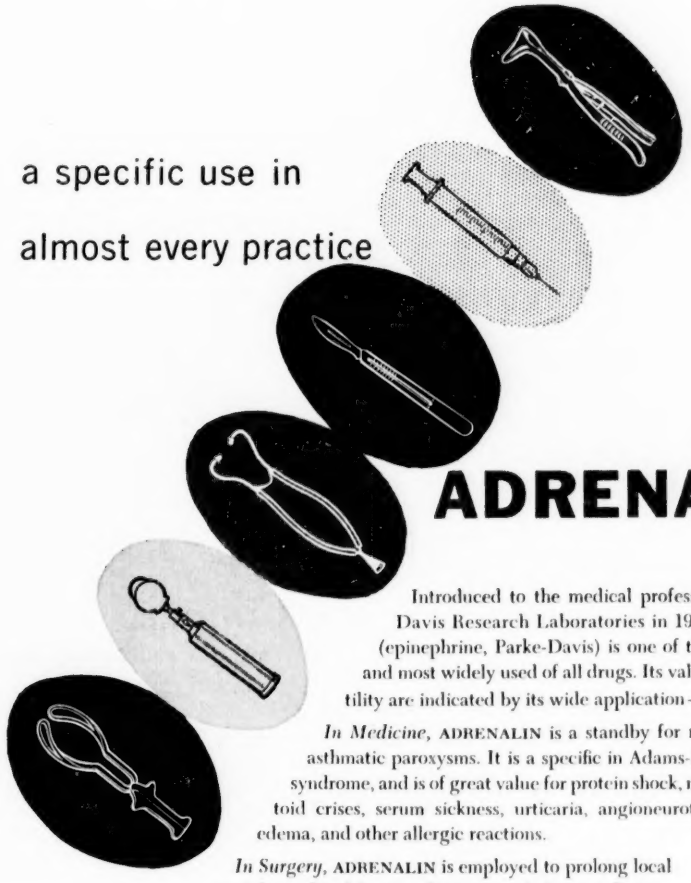

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Nitrofurantoin has a low toxicity. With oral administration it occasionally produces nausea and emesis; however, these reactions may be obviated by slight reduction in dosage. An occasional case of sensitization has been noted, consisting of a diffuse erythematous maculopapular eruption of the skin. This has been readily controlled by discontinuing administration of the drug. Animal studies, using large doses administered over a prolonged period, have revealed a decrease in the maturation of spermatozoa, but this effect is reversible following discontinuance of the drug. Until more is known concerning its long-term effects, blood cell studies should be made during therapy. Frequent or prolonged treatment is not advised until the drug has received more widespread study. It is otherwise contraindicated in the presence of anuria, oliguria, or severe renal damage.

Dosage.—Nitrofurantoin is administered orally in an average total daily dosage of 5 to 8 mg. per kilogram (2.2 to 3.6 mg. per pound) of body weight. One-fourth of this amount is administered four times daily—with each meal and with food at bedtime to prevent or minimize nausea. For refractory infections such as Proteus and Pseudomonas species, total daily dosage may be increased to a maximum of 10 mg. per kilogram (4.5 mg. per pound) of body weight. If nausea is severe, the dosage may be reduced. Medication should be continued for at least three days after sterility of the urine is achieved."

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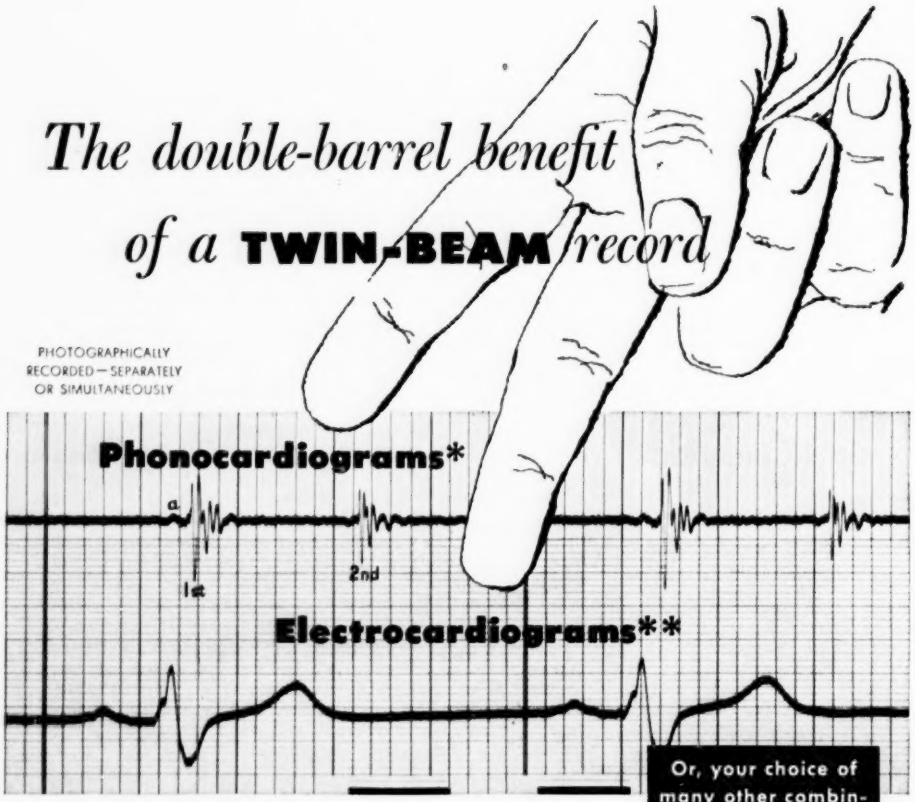

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A REFLECTION SPECTROPHOTOMETRIC STUDY OF ULTRAVIOLET ERYTHEMA AND PIGMENTATION

By M. T. JANSEN

(From the Department of Histology and Microscopic Anatomy, State University, Utrecht, The Netherlands)

(Submitted for publication November 25, 1952; accepted June 24, 1953)

Skin reactions to ultraviolet radiation have been studied several times by making histological preparations at different intervals (1, 2, 3) but this method, though indispensable, needs amplification by less destructive procedures, *e.g.*, by spectrophotometry.

METHODS AND MATERIALS

It has been established that the normal skin reflection measured on consecutive days shows considerable fluctuation, mainly because of differences in the width of the blood vessels and in the degree of oxygenation of the blood. This fluctuation precludes the prolonged observation of slight degrees of erythema or of other alterations in the color of the skin, if reference is made to a standard as is done in most spectrophotometric procedures.

In order to overcome this difficulty we have chosen to compare the experimental skin area with closely adjacent normal areas of the skin by recording successively the reflection of normal and altered areas: An optical system projects an image of the exit slit of a monochromator onto the skin, *i.e.*, it illuminates a small rectangular area of the skin with monochromatic light of any desired wave length. The slit image, which measures 3 mm. in height and 0.2 mm. in breadth, is made to scan at a speed of 2 mm. per second, a skin area of 3 by 5 mm. (see Figure 1). The light scattered back by the skin is collected in an integrating sphere and measured by means of a vacuum photocell. The amplified photocurrent is recorded on a photographic paper strip moving synchronously with the illuminating spot on the skin. Moreover, after each sweep, zero reflection and the reflection of an invariable standard is recorded automatically.

Figure 2 gives two typical registrations obtained with different parts of the skin. In these recordings the zero lines (0) connect the zero reflection registrations whereas the lines St are on the level of the standard reflection at the wavelength used. In the left-hand recording the skin reflection line, though sloping, is straight: no local alteration in skin reflection has been recorded by the scanning slit image. In the right-hand registration, however, the depression in the skin line clearly marks the presence of an area of reduced reflection. To what extent these recordings can be used in the analysis of ultraviolet irradiation effects will be discussed in the next section.

The investigation, for which the method described in the foregoing section was developed, aimed at the solution of the following problem: are erythemata (and pigmentations) yielded by ultraviolet irradiation of 300 mμ exactly like those caused by 257 mμ? It has been generally assumed that there is only one erythematogen mechanism in the ultraviolet range below 300 mμ, the "dip" in the action spectrum at 280 mμ being caused by the absorption of the horny layer. Recently, Blum and Terus (4) have suggested a modification of the 300 mμ erythema by a direct action of the rays on the capillaries themselves, whereas Rottier and Mullink (5, 6) advocate the existence of two entirely different photochemical mechanisms elicited by 300 and 257 mμ, respectively.

Precise comparison of the two kinds of erythema (and pigmentation) should, in our opinion, be of great value in this controversy as well as adding to our knowledge of skin physiology in general.

For this purpose small areas (1 by 5 mm.) of moderate erythema were generated by irradiation with 257 and 302 mμ, respectively (mercury arc, quartz spectrograph). All irradiations were performed on the forearm of one dark blonde male subject. The reflection of the irradiated areas and of the adjoining normal skin was recorded at regular intervals after the irradiation.

As regards the choice of the wave lengths at which to perform the measurements, we were guided by the known peculiarities of the transmission spectra of the pigments to be expected, *i.e.*, primarily hemoglobin (7) and melanin (8, 9) (Figure 3).

Though it is obviously important to choose the wave

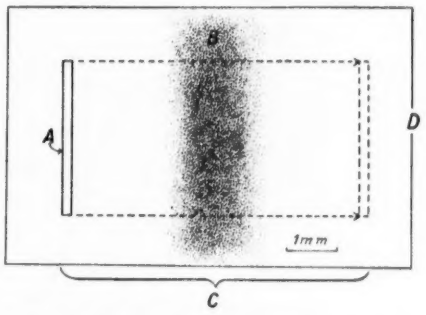

FIG. 1. DIAGRAM OF INVESTIGATED SKIN AREA

A: scanning spot of light projected onto the skin surface; B: colored skin area; C: width of skin area scanned by A; D: border of the window in the integrating sphere to which the skin is applied.

FIG. 2. TYPICAL CURVES OBTAINED WITH THE METHOD

Right: erythema or pigmentation present; left: no local coloring of the skin; 0: zero line; St: level of the reflection of the standard; Sk: normal skin reflection level (skin line).

lengths to be measured at strategical points (designated by dots) along the hemoglobin curve, the same cannot be said in the case of melanin. Note that the transmission of a melanin solution at 600 mμ is only slightly different from that at 579 mμ, whereas in the case of hemoglobin there is a great difference between the transmission values at these two wave lengths.

The two rows of curves of Figure 4 are actual registrations of the reflection of two differently colored areas of the skin, the top row referring to an erythematous area, the bottom row to a preponderantly pigmented streak. Both areas are represented by a depression in the 520 mμ curves but in the 600 and 313 mμ curves only the pigmented area is recognizable. That the erythema is not visible in the 600 mμ curve can be readily understood as blood pigment absorbs only a small fraction of the light of this wave length. The 313 mμ graph fails to record the presence of an increased amount of blood in the capillaries, as the upper layer of the epidermis forms an effective screen for this wave length. All or most of the little light of this wave length received back from the skin was reflected by the surface and scattered back by the uppermost layers and did not traverse the capillaries of the dermis. As the melanin, responsible for the depressions in the bottom row of curves, was located in the upper layers of the epidermis, its presence was also shown by the 313 mμ recording. Note the different shapes of the depressions at 600, 520, and 313 mμ, which might suggest different distributions of the light absorbing substances. Yet, this difference is more plausibly explained by the fact that in the epidermis the penetrating power of light diminishes with decreasing wave lengths. In the case of 600 mμ the bulk of the light thrown back by the skin comes from the scattering collagenous fibers of the dermis, whereas we have seen that light of 313 mμ is preponderantly reflected by the uppermost layers. As in the case of the curves of Figure 4 (II), the screen of pigment was located in the upper layers of the epidermis; the 313 mμ light, being scattered back from the same plane, can be considered to trace the distribution of the pigment most truly, whereas the depression in the 600 mμ recording will necessarily give a distorted picture of the distribution of pigment in the area. These considerations have been borne out by experiments with neutral grey screens of accurately known geometry applied to the surface of the skin.

RESULTS

With these observations in mind we now turn to the results of our measurements.

1. Reflection spectra of ultraviolet erythema and pigmentation

The essential feature of our method lies in the comparison of the altered skin with normal skin.

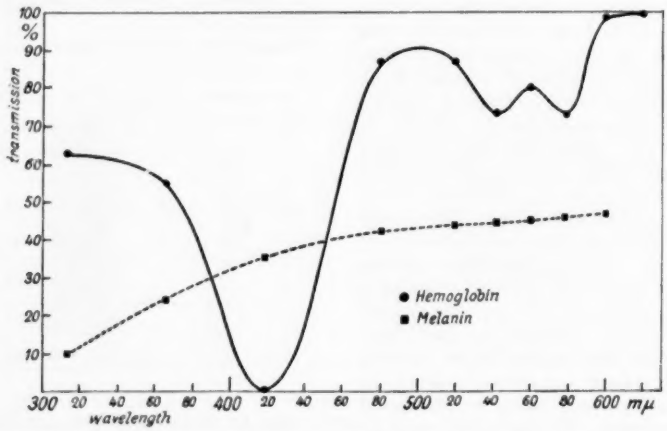

FIG. 3. TRANSMISSION SPECTRA OF 75.6 PER CENT OXYGENATED HEMOGLOBIN IN A CONCENTRATION AND LAYER THICKNESS COMPARABLE TO A BLOOD FILM OF 10 μ THICKNESS (DRABKIN AND SCHMIDT (7)) AND OF MELANIN "IN ALKALINE SOLUTION" (EDWARDS, FINKELSTEIN, AND DUNTLEY (9))

Accordingly, the measured values of reflection in the erythematous and pigmented areas have been plotted as percentages of "normal skin reflection" (*i.e.*, the mean of the reflection values of the "normal" skin areas on both sides of the area under investigation [1]).

In Figure 5, the reflection of the center of an irradiated area (257 mμ), at different intervals after the irradiation, has been plotted in this manner. The one day curve apparently shows the presence of an excess of blood pigment in the irradiated area: erythema. The curve resembles the transmission curve of Figure 3, but it cannot be considered as such because of the scattering and absorption of light in the superficial layers of the skin. These effects cause a distortion of the transmission curve which is greatest at the short wave length end of the spectrum.

On the first day after irradiation melanin is not increased as reflection at 600 and 620 mμ has remained normal. After four days, however, the influence of increased melanin pigment in the irradiated area on the reflection spectrum is indicated by the lowering of the 600 and 620 mμ values and of the whole curve especially towards the shorter wave lengths. After eight days the greatest difference from the foregoing curve is found in the short wave length region.

A further increase in pigmentation cannot be responsible for this, as the values at 600 and 620 mμ have remained unaltered. Without reverting to the assumption of an alteration of the absorption

[1] By comparing the observed normal skin values with the standard reflection values we have found a normal reflection spectrum not essentially different from those observed by other workers in the field.

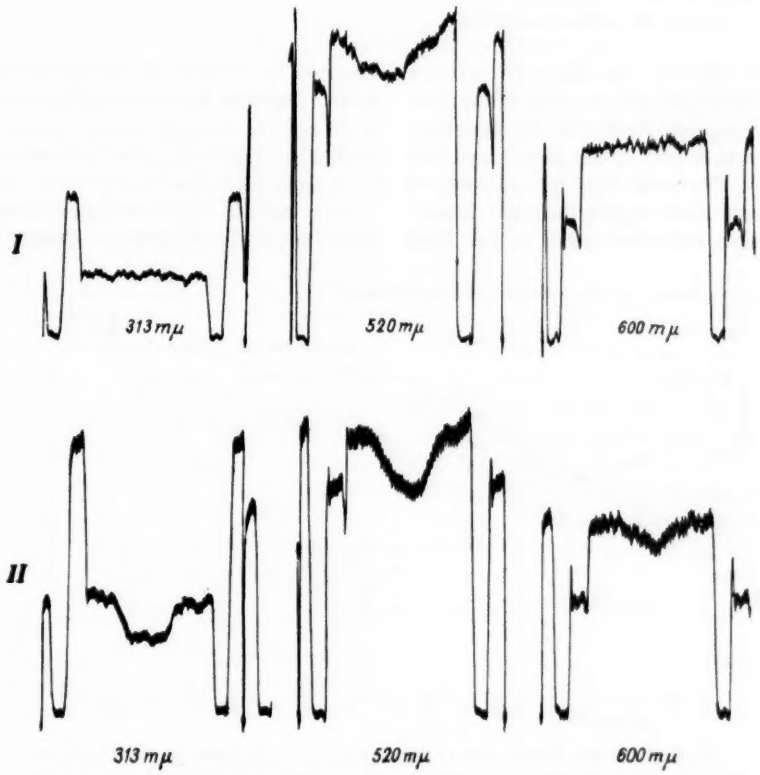

FIG. 4. SELECTED TYPICAL CURVES ILLUSTRATING THE DIFFERENCE BETWEEN THE SPECTRAL REFLECTIONS OF ERYTHEMATOUS (TOP ROW) AND PIGMENTED AREAS (BOTTOM ROW)
The numbers refer to the wave lengths at which the curves were recorded.

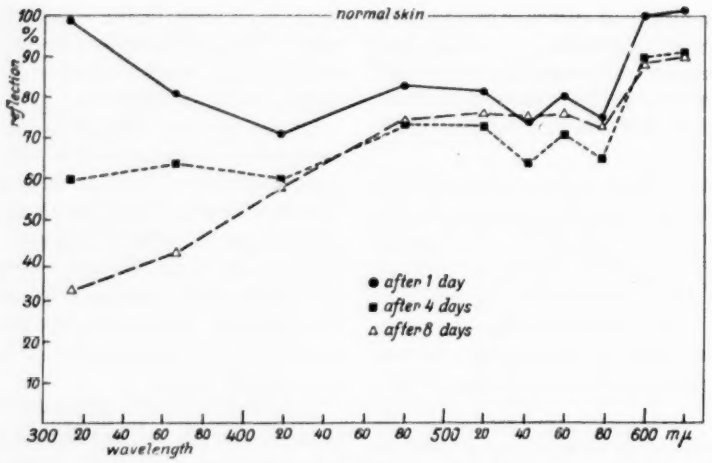

FIG. 5. SPECTRAL REFLECTION OF THE CENTER OF AN AREA OF THE SKIN IRRADIATED WITH 257 mμ ULTRAVIOLET LIGHT, EXPRESSED IN PERCENTAGES OF THE NORMAL SKIN REFLECTION

See text for further explanation.

spectrum of the pigment, the difference between the fourth and eighth day curves can be interpreted as follows: the melanin formed in the first days after irradiation is located in the deeper layers of the epidermis. Thus, the low transmission of melanin at short wave lengths does not greatly influence the skin reflection curve as has been discussed in the case of hemoglobin in the capillaries. Pigment, however, migrates to the surface of the epidermis in the course of a few days. Accordingly it gradually gains influence on the short wave length reflection values of the area.

Note that after eight days the absorption of hemoglobin is still recognizable though the 418 mμ

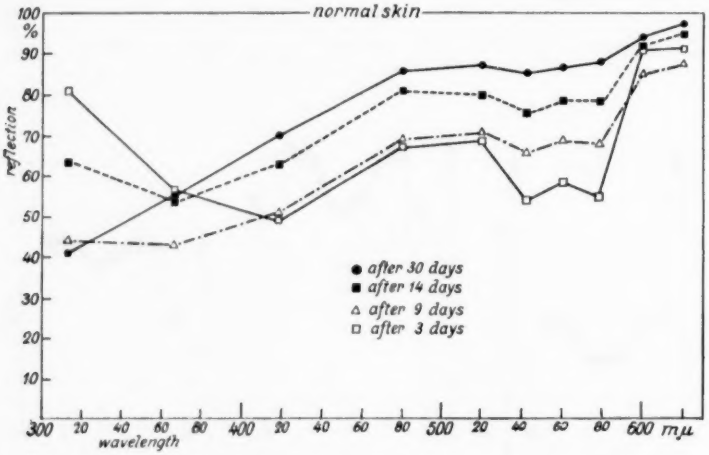

FIG. 6. SPECTRAL REFLECTION OF THE CENTER OF AN AREA OF THE SKIN IRRADIATED WITH 300 mμ ULTRAVIOLET LIGHT, EXPRESSED IN PERCENTAGES OF THE NORMAL SKIN REFLECTION

Note persistence of erythema characteristics after as many as 30 days. See text for further explanation.

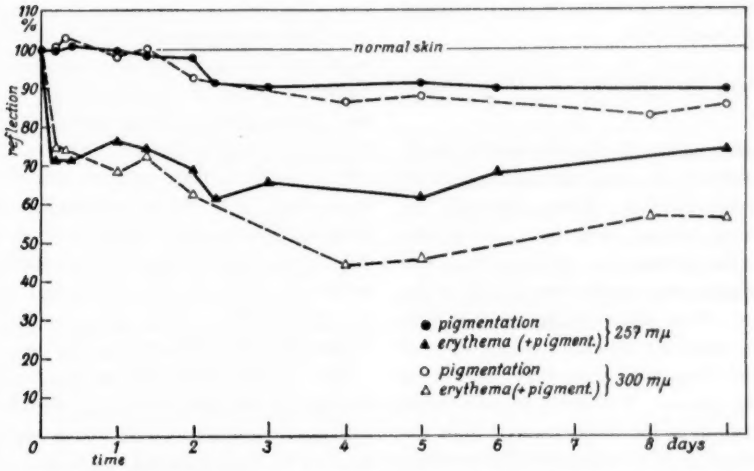

FIG. 7. SKIN REFLECTION AT 579 (ERYTHEMA + PIGMENTATION) AND 600 mμ (PIGMENTATION ONLY) IN CENTER OF AREAS IRRADIATED WITH 257 AND 300 mμ, RESPECTIVELY

Reflection is in percentages of normal skin reflection, time in days.

depression has been obscured by the melanin absorption.

Comparison of these curves with similar recordings of areas irradiated with 300 mμ instead of 257 mμ yields no significant differences.

Figure 6 shows the reflection spectra of a 300 mμ ultraviolet erythema which has been followed over a period of 30 days. The ninth and third day curves differ in the same way as do the eighth and fourth days curves of Figure 5. The differences between the curves obtained at the ninth and fourteenth days are particularly interesting, as scaling of the irradiated area took place on the eleventh and twelfth days. The curves suggest that scaling involves a loss of superficially situated pigment. The erythemal intensity, as judged by the difference of the reflection values at 600 and 579 mμ, seems not to have been greatly influenced by the scaling.

The erythema is still discernible in the curve taken as many as 30 days after the irradiation. The same has been found to be the case after 257 mμ irradiations.

2. *Mode of appearance of erythema and pigmentation after irradiation*

In Figures 7 and 8, erythemal and pigmentation values have been plotted against time. For these curves, the 579 mμ reflection values have been used to denote the degree of erythema, although, after the first few days, melanin pigment, too, will influence the reflection values at this wave length. The amount of melanin could be assessed separately with the aid of the reflection values at 600 or 620 mμ.

If one follows the course of erythema and pigmentation at daily intervals after the irradiation (see Figure 7), no differences between the results of 257 and 300 mμ irradiation are noted, provided that ultimate degrees of erythema and pigmentation are moderate and not too widely divergent.

Measurements repeated at hourly intervals, however, consistently showed the 257 mμ erythema to appear several hours earlier than the 300 mμ redness. Figure 8 gives a typical example. The

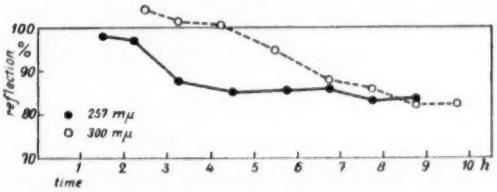

FIG. 8. DEGREE OF ERYTHEMA (AS MEASURED BY THE 579 mμ REFLECTION IN PERCENTAGES OF NORMAL SKIN REFLECTION) AT HOURLY INTERVALS AFTER IRRADIATION WITH 257 OR 300 mμ ULTRAVIOLET LIGHT

temporary increase in reflection of the irradiated area will be discussed below.

3. Distribution of the pigment in the irradiated skin areas

The information our method yields on the distribution of pigments in the area scanned should be used with extreme caution. Even a sharply delineated absorbing screen, with its borders exactly parallel to the rectangular scanning spot, will not yield a perfectly rectangular depression in the reflection curve. First, the scanning spot of light is not infinitely narrow; secondly, in the case of the skin the light does not always come back from the plane of the screen. Furthermore, the mean depth of penetration of the backscattered light varies with the wave length; moreover, the position of the two screens considered (*viz.*, the distended capillaries and the augmented pigmentation) may be different, too. The latter difficulty cannot be circumvented but we can cope with the former by comparing recordings taken at one wave length only.

In Figure 9 of three recordings of one and the same area (one, five, and eight days after irradiation with 300 mμ) the depressions in the skin lines have been superimposed. The recordings had been made at 520 mμ, as the skin reflection at this wave length is affected moderately by both erythema and pigmentation. After one day, erythema will be solely responsible for any depression in the curve, whereas pigmentation will dominate after eight days. Yet, the curves indicate an essentially identical distribution of the colored matter irrespective of the interval between irradiation and registration, *i.e.*, irrespective of whether it be erythema or pigmentation. The same result has been obtained after irradiation with 257 mμ.

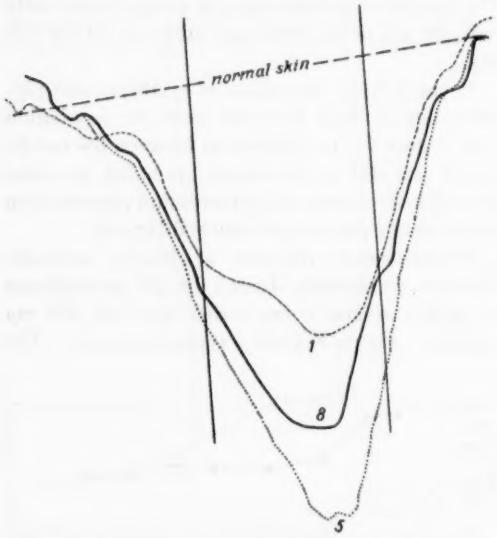

FIG. 9. SUPERIMPOSED RECORDINGS OF AN IRRADIATED (300 mμ) SKIN AREA TAKEN WITH 520 mμ AT INTERVALS OF ONE, FIVE, AND EIGHT DAYS AFTER THE IRRADIATION

Distance between vertical lines indicates breadth of irradiated area (1.5 mm.).

The vertical lines indicate the position and the breadth of the area exposed to the ultraviolet light during irradiation. The curve suggests an extension of the erythemal reaction (and of the pigmentation) beyond the borders of the irradiated area, but we cannot be certain on this point, as we do not know to what extent the scattering of light may blur the contours of the colored area. The shapes of the curves of different identically irradiated areas of the skin differed somewhat. Therefore, we could not compare with exactness the distribution of erythema and pigmentation of areas irradiated at 257 mμ with those exposed to 300 mμ.

DISCUSSION

1. The described method allows comparison, by spectrophotometric means, of very small skin areas (approximately 1 by 4 mm.) with closely adjacent normal skin areas or with a standard. Further reduction of the dimensions of the spot of light will not enhance the resolving power of the method, as the penetration of the skin by the light and the minute irregularities of the skin surface are limiting factors, which are canceled out by the length of the scanning spot.

2. In interpreting the observed phenomena, we have assumed two pigments to increase in quantity as the result of ultraviolet irradiation. That most of the facts can be explained on this assumption in a semi-quantitative manner does not imply of course that subtler methods would fail to detect other light-absorbing substances involved in the reactions.

The results obtained can be summarized as follows:

Erythema is the first constant result of appropriate ultraviolet doses; the 257 mμ erythema is spectrophotometrically identical with the 300 mμ erythema. The latter, however, tends to appear later than the former. In both cases, the erythema is shown to persist much longer than is generally assumed. In fact, for the duration of our experiments (45 days) no visible result of ultraviolet irradiation has been found in which erythema was not detectable. Recently, Schulz (10) obtained similar results.

No differences in the modes of appearance and in the spectrophotometric characteristics of 257 and 300 mμ pigmentations have been found. Pigmentation always followed and never preceded erythema. There is a close correlation between the areas of erythema and pigmentation.

We found no evidence for the assumption of Keller (1) and Miescher (2), based on histological findings, that the first stages of visible pigmentation can be accounted for solely by a migration of the pigment already present. On the contrary, our findings indicate an early increase of melanin and subsequent migration of the pigment.

Thus, the question originally posed, *i.e.*, "Are erythemata and pigmentations caused by 300 mμ exactly like those following irradiation with 257 mμ," should be answered affirmatively.

It would seem that the photochemical processes elicited by the two wave lengths converge to form one common stimulus to the skin capillaries. The reason for the difference in speed of appearance of 300 and 257 mμ erythemata should, then, be sought in the earlier stages of the photochemical processes (Rottier, personal communication). In this connection, reference should also be made to the supposition by Blum and Terus (4), that the direct influence of 300 mμ rays on the capillaries may delay the reaction to the dilatator substance.

It is probable that there is only one stimulus for melanin production, too. This stimulus may be of the same nature as that eliciting erythema, but one should bear in mind the possibility that increased melanin production may be a sequel to erythema. The latter supposition is favored by the finding of the long persistence of erythema and of the exact coincidence of the erythematous and pigmented areas.

One might well ask whether ultraviolet irradiation invariably results in increased pigmentation. We have, in fact, come across a few instances in which, during the first few days after irradiation, a slight bleaching of the skin superimposed on the erythema is suggested by the curves. The bleaching could be localized in the epidermis, but we have not been able to find out whether in these cases the increase in reflection should be ascribed to a real diminution of pigment or to an increase of the scattering power of the epidermal layers. This phenomenon, which has also been found after local x-ray irradiation of the same subject, certainly merits further investigation.

SUMMARY

1. A method is described enabling small areas of the skin to be scanned with a narrow strip of monochromatic light. The skin reflection during scanning is recorded, and repeating the process at various wave lengths yields data on the spectral reflection of erythematous and pigmented fields in the area scanned and on the distribution of the colored matter. Narrow rectangular areas of erythema and of pigmentation caused by ultraviolet irradiation (257 or 300 mμ) are thus examined.

2. It appears that skin reactions to moderate irradiations with 257 mμ and 300 mμ, respectively, differ only in the speed of formation of the erythema, the 257 mμ erythema preceding the 300 mμ erythema of comparable "strength" by several hours.

3. Ultraviolet erythema is shown to persist for at least 45 days after irradiation.

4. A very close spatial and temporal relationship has been found to exist between erythema and pigmentation after ultraviolet irradiation.

ACKNOWLEDGMENTS

The author is indebted to Prof. Berkelbach van der Sprenkel and to Dr. Rottier for constant encouragement and valuable criticism. His thanks are also due to Miss A. de Ruiter for untiring technical assistance.

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INORGANIC PHOSPHATE RESPONSE TO INTRAVENOUS GLUCOSE ADMINISTRATION IN PROGRESSIVE MUSCULAR DYSTROPHY

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(Submitted for publication June 16, 1953; accepted July 1, 1953)

INTRODUCTION

One outstanding biochemical finding in progressive muscular dystrophy (P.M.D.) is excessive creatinuria. Much of the experimental work on the disease therefore has been centered on creatine-creatinine metabolism. The conclusion has now been reached that the creatinuria in this condition arises from a normal endogenous creatine production in the presence of impaired removal of creatine from the blood stream as a result of the severe diminution of muscle mass (1). It has been furthermore inferred that such parts of the muscles which survive, remove creatine at a normal rate. This decrease in functional muscle mass appears to be a common feature in all distinct cases of progressive muscular dystrophy.

In view of the well established role of carbohydrates as a source of energy in muscular activity, it is surprising that until now hardly any evidence has become available of a disturbance of carbohydrate metabolism in P.M.D. Abnormalities in glucose metabolism have been reported, including hypoglycemia, by McCrudden and Sargent (2, 3), reduced sugar tolerance by Janney, Goodhart, and Isaacson (4), and increased utilization of glucose by Magee (5), but more careful studies by Shank, Gilder, and Hoagland failed to confirm these results (6).

In evaluating these negative findings of oral and intravenous glucose tolerance studies, it should be kept in mind that in the process of the removal of glucose from the blood stream in fasting subjects, the capacity of the liver is probably large enough to compensate for any deficiency on the part of the musculature. However, a crude measure of the respective portions removed by these tissues seems to be possible by the simultaneous recording of inorganic serum phosphate during the glucose tolerance test. Pollack and his coworkers (7) have demonstrated that phosphate is removed from a glucose and phosphate-containing perfusion fluid by the isolated hind limb, but not by the isolated liver of the dog. Recently, the estimation of a blood glucose and inorganic phosphate curve, after an oral or intravenous glucose load, has been used for diagnostic purposes in diabetes mellitus, various forms of liver dysfunction and in the study of glucose resorption in tropical sprue (Forsham and Thorn [8], Volk and Lazarus [9], and Fourman [10]).

In the present report, this method has been used in the study of five cases of P.M.D., and the results have been compared to those obtained in normal subjects.

METHODS

Ten normal subjects (seven males and three females), and five cases of P.M.D. (three males and two females) were studied. In four patients the disease was longstanding and had progressed to various degrees of invalidism. In one case (J. v. O.), muscular weakness was not pronounced and the diagnosis was ultimately founded on the resemblance of electromyographic records with those of her brother (case W. v. O.) who is severely crippled by the disease.

All subjects were instructed to ingest liberal amounts of carbohydrates during a three-day period preceding the test. The experiments were started at 8:30 a.m. on fasting subjects, the last meal having been taken before 8 p.m. on the previous day. A 20 per cent or 40 per cent aqueous solution of the glucose was injected in the course of 5 to 10 minutes at a total amount of ½ Gm. per Kg. body weight. During the test the subjects were kept in the recumbent position and were not allowed to smoke.

Immediately before and at intervals of 15, 30, 60, 90, and 120 minutes after the intravenous administration of glucose, venous blood was withdrawn.

The blood was collected in tubes containing powdered sodium fluoride and this was immediately mixed with the sample. Two-tenths ml. of the whole blood was removed for duplicate glucose estimations, using the method of Hagedorn and Jensen (11). Thereafter, the sample was centrifuged and inorganic phosphate was estimated in the plasma by the method of Briggs (12). The slight degree of hemolysis, which occurred in some samples, apparently

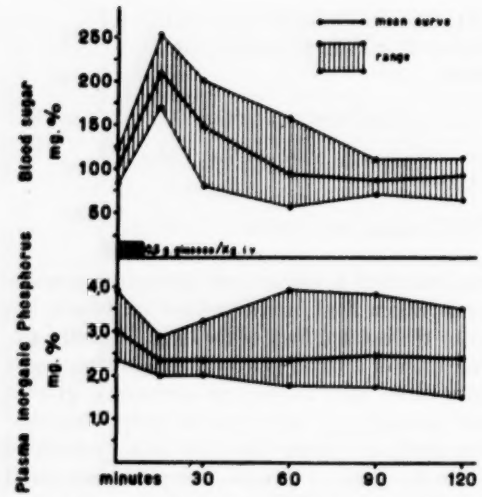

FIG. 1. CHANGES IN BLOOD GLUCOSE AND PLASMA INORGANIC PHOSPHORUS AFTER A STANDARD I.V. GLUCOSE LOAD IN NORMAL SUBJECTS

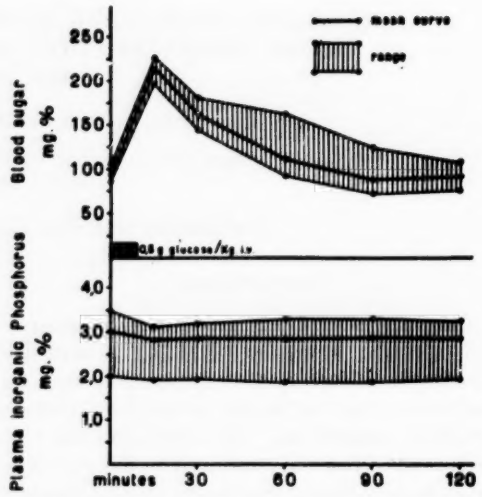

FIG. 2. CHANGES IN BLOOD GLUCOSE AND PLASMA INORGANIC PHOSPHORUS AFTER A STANDARD I.V. GLUCOSE LOAD IN PATIENTS WITH PROGRESSIVE MUSCULAR DYSTROPHY

did not influence the results, presumably because of effective inhibition of breakdown of cellular organic phosphates by the fluoride.

RESULTS

The data obtained with normal subjects are depicted in Figure 1. The initial value and the lowest value recorded at any time during the test of inorganic plasma phosphate are given for each individual case in Table I. The mean of the percentage maximum decrease of the normal individuals (28 per cent) agrees well with that given by Forsham and Thorn (8), *i.e.*, 25 per cent for 20 normal subjects. The results of these authors are not wholly comparable to ours because they infused the glucose over a 30-minute period.

In the cases of P.M.D., the mean glucose tolerance curve (Figure 2) is not significantly different from the normal. It should be mentioned that one patient (Case v. D.) showed a definitely delayed glucose response, but in the four others, a perfectly normal glucose curve was obtained.

The fall in plasma inorganic phosphate was poor in all patients and the mean maximum fall was only

TABLE I

Initial values and minimal values of plasma inorganic phosphorus during i.v. glucose tolerance test in normal and diseased subjects

	Normal subjects				Cases of P.M.D.		
Subject	Initial value mg. %	Lowest value mg. %	Maximal fall in % of initial value	Case	Initial value mg. %	Lowest value mg. %	Maximal fall in % of initial value
W ♂	3.70	2.80	24	D ♂	2.08	1.81	13
P ♀	2.96	1.97	33	B ♀	3.06	2.71	11
G ♂	3.90	3.25	17	A ♂	3.05	2.82	8
J ♂	2.55	1.49	42	O ♂	3.43	3.15	8
J ♀	2.36	1.99	16	O ♀	3.43	3.01	12
K ♂	3.43	1.83	47				—
Z ♂	2.99	2.20	26			Mean (5)	10
B ♂	2.58	1.82	29				
R ♂	2.96	2.06	30				
V ♀	2.77	2.23	20				
			—				
		Mean (10)	28				

10 per cent. The difference between this value and the corresponding one for the normal subjects (28 per cent) is highly significant ($P < 0.001$) with no overlapping between the two groups.

DISCUSSION

The decreased phosphate response to glucose injection in the cases of P.M.D. is very probably a reflection of a diminished phosphorylation in the muscle tissue. This in turn could be regarded as the result of the decrease in functional muscle mass. Increased urinary elimination of the injected glucose cannot be responsible for these findings, because of the normal blood glucose curves. When checked in two cases, the glucosuria was found to be less than 2 per cent and 3 per cent of the total dose administered, respectively. Our data do not provide evidence for a correlation between the amount of remaining intact muscle tissue, *e.g.*, the severity of the disease and the degree of impairment of the phosphate response. One completely crippled case (v. D.) showed a maximum fall of 13 per cent, while in another very light case (J. v. O.) a maximum fall of 12 per cent was recorded. Furthermore, it is not known to what extent the amount of muscle tissue or of muscle function must decrease in order to be detected as a decreased phosphate response.

On the other hand the possibility remains of a specific biochemical lesion in the diseased muscle, involving a block somewhere in the glycolytic chain of reactions. However, most of the enzymatic and chemical changes reported can be accounted for by relative increase of collagenous material and fat (Nevin [13], Reinhold and Kingsley [14], and Hoagland [15]), and the few indications of primary changes (Hoagland [15], and Dreyfus, Schapira, and Schapira [16]) are in need of confirmation.

The decreased phosphate response reported here should, therefore, be regarded as another manifestation of the diminution of functional muscle mass, although it is realized that similar studies in other forms of muscle disease are still required to support this opinion.

SUMMARY

Variations in plasma inorganic phosphate levels in response to intravenous administration of glucose have been studied in normal subjects and in five cases of progressive muscular dystrophy. The fall in inorganic phosphate was significantly less in the patients.

ACKNOWLEDGMENTS

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TWO CASES OF POSTURAL HYPOTENSION SHOWING A DEFICIENCY IN RELEASE OF NOR-EPINEPHRINE AND EPINEPHRINE

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(Submitted for publication April 6, 1953; accepted July 23, 1953)

Of the two forms of orthostatic hypotension, one, the *arterial orthostatic anemia* or hypotonic regulatory disturbance (1), is characterized by an increase in the pulse rate and a fall of the systolic blood pressure, with or without a rise in the diastolic pressure, upon change from the recumbent to the erect posture. These circulatory reactions are due to an increased activity of the sympathetic nervous system, which normally compensates for the hydrostatic displacement of blood due to the changes in position. In the second form, the *postural hypotension* or hypodynamic regulatory disturbance, this reaction of the sympathetic system to changes in posture is absent. The result is a momentary fall of the systolic as well as the diastolic blood pressure during a change from the recumbent to the erect posture, the pulse rate being maintained at a constant level (Figure 1).

Postural hypotension was described for the first time in 1925 by Bradbury and Eggleston (2). It is a rather rare condition, of which Springarn and Hitzig (3), in 1942, found only 50 cases described in the literature. It is supposed to be due to organic lesions in the brain stem or spinal cord, following encephalitis, encephalomalacia and tumors, tabes dorsalis, syringomyelia and hematomyelia (4–6). A similar condition may also occur after extensive sympathectomies (7–9). Hammarström and Lindgren (10) made histological studies of the central nervous system in a case of postural hypotension and found lesions located in the right internal capsule, the cerebellum, the occipital lobes, and the cortex of the lateral parts of the hemispheres of the cerebrum.

In the present study of two cases of postural hypotension, the authors determined the daily urinary excretion of the catechol amines, epinephrine and nor-epinephrine. The urinary output of epinephrine after intravenous administration of insulin, which increases the output by ten-fold in healthy subjects (11), was also studied. In one of the cases, the urinary excretion of catechol amines was followed during an intravenous infusion of nor-epinephrine, as well as after a subcutaneous injection of histamine.

EXPERIMENTAL

Methods

The catechol amines in urine were determined according to the methods of Euler (12), and Euler and Hellner (13). The procedure consists, in principle, of selective adsorption of the catechol amines in the urine on aluminum hydroxide after acid hydrolysis. After elution, the adsorbed catechols are tested on the cat's blood pressure and on the hen's rectal caecum, permitting estimation of epinephrine and nor-epinephrine separately.

Insulin tolerance test. The patients were kept fasting over-night and given 0.1 I.U. of insulin per Kg. body weight intravenously at 8 A.M. Samples of capillary blood were taken 10, 20, 30, 45, 60, 75, 90, 120, 150, and 180 minutes after the injection. The blood pressures and pulse rates were determined at regular intervals. The patients' reports on hypoglycemic symptoms were noted. The urine was collected from a period of approximately two hours before (control) and three hours after the injection of insulin.

Intravenous infusion of nor-epinephrine. The ability of intravenously infused nor-epinephrine to pass through the kidneys into the urine was studied in one of the present cases (Case 1). In each of two experiments, 400 μg. of dl-nor-epinephrine (approximately equivalent to 200 μg. of the levo-compound) was administered intravenously as a constant infusion during 50 to 90 minutes. The urine was collected for two hours before (control), as well as two hours after, the start of the infusion. Epinephrine and nor-epinephrine were determined according to the methods described above. Previous experiments by Euler and Luft (14) have shown that 1.5 to 3.3 per cent of the infused amounts of nor-epinephrine are excreted during the time of infusion. Similar results were obtained by Goldenberg (15).

Effect of histamine on the excretion of catechol amines. A preliminary study was made of the effect of histamine in releasing epinephrine from the chromaffine cells (see Dale, [16]). A dose of 0.5 mg. of histamine dihydrochloride

was given subcutaneously to ten healthy subjects and twice to Case 1 of the present study. The urine was collected for two hours before (control) and three hours after the injection and submitted for analyses of epinephrine and nor-epinephrine.

Case Reports

Two cases were studied. They have been reported earlier by Luft and Sjögren (17).

Case 1. Unmarried man, 47 years old. He had rheumatoid arthritis in 1913. The first symptoms of his present illness appeared in 1938 and consisted of tiredness and dizziness, most pronounced in the mornings and during warm weather. He lost the ability to perspire. The libido disappeared. He often fainted, especially when he had to stand upright for some time. When examined in 1946 he had been almost completely incapacitated for the last year. Body weight, 57 Kg., body length 176 cm. No abnormal pigmentations; normal findings on neurological examination. No change in pulse rate or blood pressure was obtained when pressure was applied to the carotid sinus. Physical examination of the testicles and the prostate gland was normal. There were no clinical signs of adrenal cortical insufficiency. BMR + 1 per cent. Normal findings on liver puncture. Glomerular filtration rate (inulin) 99 ml. per minute, renal plasma flow (diodrast), 399 ml. per minute. Water load test (1000 ml.) showed a normal ability to dilute, concentrate and excrete. Cutler's test was normal. X-ray examinations of heart, lungs, kidneys, adrenal regions, stomach, intestines, and sella turcica were normal. Wasserman's reaction was negative. No orthostatic phenomena were observed on electrocardiogram.

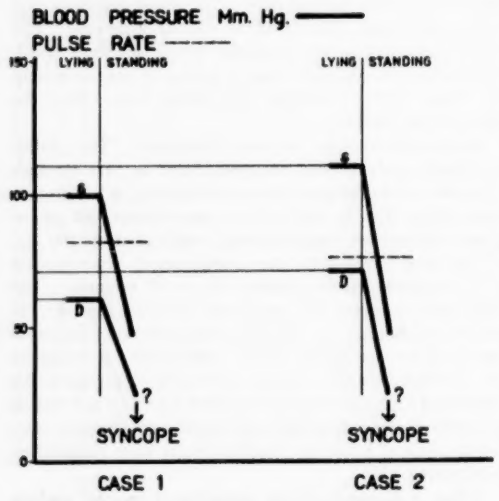

FIG. 1. CHANGE IN SYSTOLIC AND DIASTOLIC BLOOD PRESSURE DURING ORTHOSTATIC TESTS IN TWO CASES OF POSTURAL HYPOTENSION

Blood pressure (Figure 1): in the morning at rest 90–120/50–70 mm. Hg; when standing up an immediate fall of the systolic pressure to 50–60 mm. Hg was observed, while the diastolic pressure fell below 30 mm. Hg and could not be accurately measured. The pulse rate remained unchanged. After the subcutaneous administration of 0.5 mg. of ergotamine tartrate the pressure was 175/125 mm. Hg while recumbent, and 100/60 mm. Hg when standing. A similar effect was obtained with epinephrine and ephedrin.

After subcutaneous administration of 1 mg. of dl-nor-epinephrine, the blood pressure in erect posture was kept normal for several minutes.

Case 2. Married woman, born 1895. Menopause in 1946. Symptoms of present illness date from 1946 and consist of extreme fatigue, gain in weight, decreased tolerance to cold, constipation and loss of the ability to perspire. Since 1947 she had syncopal attacks which have increased in frequency. Since 1948 she fainted when she tried to walk for more than a few steps. The secondary hair growth was normal, and she had no abnormal pigmentations. There were no clinical signs of adrenal cortical insufficiency or hypothyroidism. Normal findings were present on neurological examination. Electrocardiogram at rest was normal, and there were no orthostatic changes in erect posture. BMR:—11 per cent. Glomerular filtration rate (inulin) was 80 ml. per minute and renal plasma flow (diodrast) 440 ml. per minute. X-ray examinations of sella turcica, heart, lungs, intestines and stomach were normal; the gallbladder contained several stones.

In 1951, the patient had severe hematuria, and later the same year was operated on for a hypernephroma of the right kidney. No metastases were found at the operation or afterwards.

Blood pressure (Figure 1) in the morning at rest was 100–130/60–85 mm. Hg. On changing to the erect posture there was an immediate fall of the systolic pressure to 40–50 mm. Hg, while the diastolic pressure fell below 30 mm. Hg. The pulse rate remained unchanged. After administration of 0.5 mg. of ergotamine tartrate, subcutaneously, the reclining pressure was 180/115 while the standing pressure was 75/55 mm. Hg.

When one mg. of dl-nor-epinephrine was administered subcutaneously, the blood pressure with the patient in the erect posture remained normal for several minutes. When one mg. was given in a suspension in oil with ascorbic acid as a stabilizer, it was kept normal or even elevated for about one hour. However, palpitations, oppression, and a feeling of apprehension made this treatment less attractive.

RESULTS

Resting secretion

The daily excretion of epinephrine and nor-epinephrine in our two cases of postural hypotension as well as in healthy subjects are given in Table I. Table II shows the values of excretion

TABLE I

Daily urinary excretion of epinephrine and nor-epinephrine in two cases of postural hypotension

Case no.	Epinephrine in urine μg./24 hours	Nor-epinephrine in urine μg./24 hours	Epinephrine in per cent of total catechols in urine
1	2.0	13.0	13
	2.8	5.8	33
2	1.2	3.0	28
	0.92	5.3	14
	0.80	3.6	18
	0.62	2.7	19
	0.93	4.2	18
Healthy subjects	3–6	20–40	10–20

figures during a control period before administration of insulin. The control figures earlier reported by Euler and Luft (11) and included in Table II are somewhat high since these subjects arrived at the laboratory in the morning which involved a certain degree of activity. In hospitalized patients the control excretion figures should be about 2 mμg. per minute of epinephrine and 20 mμg. per minute of nor-epinephrine.

As seen in Table I, the catechol amine output in the cases of postural hypotension was low when compared with the normal values. This holds true especially for nor-epinephrine. The excretion of epinephrine during the control period (before the injection of insulin), 0.8 to 2.8 mμg. per minute, and 0.62 to 2.8 μg. per 24 hours, was low but possibly within the normal range for an individual at rest. The excretion of nor-epinephrine was, however, only 2.0 to 7.3 mμg. per minute during the control period, and 2.7 to 13 μg. per 24 hours.

Insulin test

While insulin in normal subjects increased the epinephrine output by about 50 mμg. per minute (11), it did not change the excretion of catechols significantly in the two present cases. The largest increase of epinephrine, from a control value of 1.3 to 7.7 mμg. per minute, is of doubtful significance.

The pulse rate was unchanged throughout the tolerance test in three out of four studies. The blood pressure showed a continuous fall during the initial 60 to 90 minutes after the insulin injection. The initial values and the lowest values obtained are recorded in Table II. In all instances the blood pressure then rose to the initial level.

TABLE II

Effect of insulin on the urinary excretion of epinephrine and norepinephrine, the pulse rate, blood pressure and blood sugar in two cases of postural hypotension

Cases			Epinephrine in urine μg./min.			Nor-epinephrine in urine μg./min.			Epinephrine in per cent of total catechols			Pulse rate per min.		Blood pressure mm. Hg		Blood sugar mg. per cent		
No.	Age	Sex	Control period	Insulin period	Diff. X100	Control period	Insulin period	Diff. X100	Control period	Insulin period	Diff.	Control period	Insulin[1] period	Control period	Insulin[2] period	Control period	Insulin[2] period	Change in per cent
1	47	M	0.0013	0.0077	+0.64	0.0025	0.0096	+0.71	34	45	+11	75	76	150/95	120/75	99	36	−64
			0.0028	0.0022	−0.06	0.0073	0.0024	−0.49	27	46	+19	68	74	155/100	115/75	105	38	−64
2	57	F	0.0012	0.0020	+0.08	0.0065	0.0027	−0.38	16	43	+27	72	73	125/85	60/40	105	45	−57
			0.0008	0.0010	+0.02	0.0020	0.0026	+0.06	29	28	− 1	68	80	140/100	65/45	100	30	−50
Healthy subjects			0.0055± 0.0009	0.055± 0.007	+4.9± 0.7	0.040± 0.004	0.026± 0.004	−1.3± 0.6	12.0± 1.6	67.5± 3.5	55.5± 3.8							

[1] Denotes the highest values obtained.
[2] Denotes the lowest values obtained.

In healthy subjects the insulin hypoglycemia is accompanied by an increase of the systolic and a decrease of the diastolic blood pressure as well as an accelerated pulse rate (11, 18). These and other changes would be best explained by an increased secretion of epinephrine (11, 19). The absence of circulatory signs in the two cases of postural hypotension after insulin is in accord with the lack of increase in epinephrine excretion observed in the present study.

The decrease in blood sugar after insulin administration was pronounced in these cases. It returned to the initial level except for experiment 2 (case 1), where glucose had to be given after 40 minutes to get the patient out of a comatose state. Despite this, the blood sugar did not return to the initial level for the remainder of the period.

The subjective reaction to insulin which appeared after some 10 to 15 minutes, mainly consisted of sleepiness and drowsiness. During a large part of the insulin period the patients were asleep and woke up only when the blood samples were taken. In three insulin tolerance tests the symptoms gradually disappeared during the last hour. In study 2, the patient went into coma with convulsions (see above). In no instance did there appear sweating, palpitations, or an agitated state, mostly observed in healthy subjects during an insulin tolerance test. In the cases of postural hypotension, there was more of a "silent fading out."

During the infusion of 400 μg. of dl-nor-epinephrine to case 1, 0.6 and 1.0 per cent, respectively, were excreted in the urine in two studies, which may be regarded as approximately normal (14). The amount of nor-epinephrine excreted increased from 2.5 to 14 and in the second study from 3.4 to 27 mμg. per minute. At the same time epinephrine increased from the control values of 2 and 0.5 to 9 and 5 mμg. per minute, respectively.

In a preliminary study the injection of 0.5 mg. of histamine dihydrochloride subcutaneously in ten healthy subjects evoked an increase in the excretion of epinephrine from a mean control value of 2.3 mμg. per minute to 7.5 during the first and 15 during the second hour after the injection. The increase in the excretion was thus 5.2 and 13 mμg. per minute. All subjects demonstrated a marked reaction with flushing of the face and complained of headache for some minutes up to one hour. When the same histamine dose was given to case 1, the patient did not show any such reaction at all. The excretion of epinephrine showed a hardly demonstrable increase, from a control value of 0.3 to 0.6 and 0.9 mμg. per minute during the first and second hour after the injection, which means an increase of 0.3 and 0.6 mμg. per minute, only.

The spontaneous excretion of nor-epinephrine was not significantly altered by histamine.

DISCUSSION

The diagnosis of postural hypotension in the present two cases seems to the authors to be beyond question. They demonstrated all the characteristic symptoms of the disease and did not reveal any signs of other disturbances in the circulatory system or in the endocrine glands.

The daily excretion of epinephrine and nor-epinephrine was low in both cases, which may well be connected with the inability of the sympathetic nervous system to react to changes in posture. In this connection it should be noted that subcutaneous administration of nor-epinephrine relieved the patients of all signs of hypotension.

In general, a decreased excretion of catechol amines may be due to: a) An inability of catechol amines released to pass through the kidneys into the urine; b) an inability of the adrenergic nerve endings and the chromaffine cells to react to stimuli and to produce or release the hormones; and c) an inability of the centers to react to physiological stimuli and to send impulses leading to a release of catechol amines from the adrenergic nerve endings or the chromaffine cells.

As to the first point, intravenous administration of nor-epinephrine to one of our cases gave rise to an increase in the excretion of nor-epinephrine that was only a little below the increase obtained under similar conditions in healthy subjects (14). This shows the intact ability of the kidneys to excrete circulating nor-epinephrine with the urine.

As to the second point, the studies with histamine have demonstrated a decreased ability of the adrenal medulla to release epinephrine on direct stimulation. Since about 80 per cent of the total catechols of the adrenal medulla is epinephrine and only 20 per cent nor-epinephrine (20), the effect of histamine will be reflected mainly on the excretion of epinephrine. Whether the lack of histamine response is due to lack of medullary epinephrine or other causes cannot be decided.

The failure of insulin hypoglycemia to evoke an increased secretion of epinephrine in the case studied might then be explained by an inability of the adrenal medulla to produce catechol amines, although functional damage to the regulating centers or blocking of the efferent sympathetic pathways has to be considered as a cause. The occurrence of injuries in the central nervous system in cases of postural hypotension suggests the latter possibility.

Clearly the low and physiologically inadequate secretion of nor-epinephrine from the adrenergic nerve endings could be explained along the same lines, *i.e.*, failing production or absence of secretory impulses. Catechol amine analyses of organs might give some information on this point.

A decreased ability of the adrenergic nerve endings to form nor-epinephrine bears certain analogies to the conditions in myasthenia gravis, where the motor nerves apparently do not produce adequate amounts of acetylcholine.

SUMMARY

In two typical cases of postural hypotension, the spontaneous urinary excretion of nor-epinephrine was markedly decreased.

The normally occurring strong increase in the excretion of epinephrine after intravenous administration of insulin was lacking in these patients. The pulse rate and blood pressure did not show the changes, during insulin hypoglycemia, typical of the secondary hyperadrenalinemia in normal subjects. The hypoglycemic symptoms were different from those observed in healthy subjects after insulin administration.

Infused nor-epinephrine was excreted in a normal way when tested in one of the patients. A complete refractoriness to histamine was noted, both as regards subjective signs and with regard to the normally occurring increase in epinephrine excretion in the urine.

The possible mechanisms of the diminished catechol amine excretion in these cases is discussed.

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FILTER PAPER ELECTROPHORESIS OF HUMAN HEMOGLOBIN

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Since the observations of Pauling, Itano, Singer, and Wells on the presence of abnormal hemoglobin in the blood of patients with familial anemias, there has been a need for a rapid and simple method for the electrophoretic study of hemoglobin (1, 2). Following the earlier work describing techniques of filter paper electrophoresis (3–7), a number of studies have been made on the use of this method for the separation of proteins. Filter paper electrophoresis does not require elaborate equipment and can be adapted for the quantitative estimation of protein patterns yielding information similar to that obtained with free electrophoresis.

Spaet (8) has recently described the identification of abnormal hemoglobins using the Durrum apparatus for paper electrophoresis (7). The present communication will outline the methods used in filter paper electrophoresis of human hemoglobin using the technique described by Kunkel and Tiselius (9). With filter paper electrophoresis it is possible to obtain both qualitative and quantitative data on mixtures of hemoglobins; the presence of an electrophoretically abnormal hemoglobin can be established, its relative concentration determined, and an elution curve may be constructed.

MATERIALS

Electrical. Power supply unit with a variable voltage over a range of about 600 volts; ammeter with a range of 0 to 25 milliamperes; ¾ inch carbon electrodes.

Filter paper. Whatman No. 3 MM.

Staining. Stock solution of 1 per cent brom-phenol-blue in ethyl alcohol, saturated with mercuric chloride, diluted 1 : 50 for use with 0.5 per cent acetic acid. Rinsing solution of 0.5 per cent acetic acid.

Glassware. One pair 8 by 30 centimeter glass plates for preparative or qualitative work; one pair 20 by 45 centimeter glass plates for quantitative work; one pair 1 liter rectangular battery jars for filter paper bath in preparative work; one pair 4 liter containers with covers for filter paper bath in quantitative work; glass cylinders for staining and rinsing.

[1] John and Mary R. Markle Scholar in Medical Science.

Buffer. Veronal buffer 0.025 M, pH 8.6, ionic strength 0.025. This buffer, made by dissolving 0.69 gram veronal and 4.38 grams of sodium veronal in one liter of distilled water, is used for filter paper baths, dilution of hemoglobin and dialysis of hemoglobin.

Sealing. Silicone stopcock grease for establishing a non-wettable surface on glass plates next to filter paper; Apiezon compound to form airtight seal along exposed edges of glass plates.

Hemoglobin preparation. 0.9 per cent sodium chloride, toluene, carbon monoxide gas.

Quantitative reagents. For tyrosine group determination, 12.5 per cent sodium carbonate, 0.1 per cent copper sulphate, 0.15 N sodium hydroxide, Folin reagent (10). For dye-elution technique, 0.1 per cent sodium hydroxide. Use of these reagents in association with the electrophoretic analysis of other proteins has been previously described (9).

METHODS

Hemoglobin for use in these studies was prepared by the method of Drabkin (11), and converted to the more stable carboxyhemoglobin. Some of the specimens were dialyzed before electrophoretic analysis; this was carried out at 4° C. against 0.025 M veronal buffer at pH 8.6 over a period of two to six days. A final concentration of hemoglobin of approximately 4 grams per cent was used in the runs. Dilutions of hemoglobin were made with the veronal buffer.

The two filter paper baths and the two electrode jars were filled with buffer, and preliminary equilibration of the levels of buffer in the four containers was insured by the use of a siphon which could be clamped off or removed during the run. A sheet of filter paper was cut to fit the width of the glass plates, allowing about 5 to 10 centimeters of overlap at the ends of the plates. The smoother side of the paper was selected as the face or upper side. A baseline was established midway between the two ends of the paper and sites of placement for the hemoglobin were marked along the baseline. Other data such as time of run, voltage, buffer, pH, volume and concentration were written directly on the paper. The marked filter paper was dipped in the buffer solution, then blotted for one or two minutes on a second piece of filter paper. The glass plates were polished on one side with a cloth containing a small amount of silicone stopcock grease to form a non-wettable surface next to the filter paper. The damp-dry, labeled filter paper was then placed on one of the glass plates, centering it carefully so as to give equal overlap of the paper at each end of the plate.

For the qualitative or preparative runs, 0.005 milliliter samples of a 4 per cent solution of carboxyhemoglobin were placed on a sheet cut to fit the smaller set of glass plates. Five samples could easily be run on a single sheet without crowding. After placing the samples on the paper, the second glass plate was laid directly over the lower plate. Apiezon compound was molded along the exposed edges of the glass plates to reduce evaporation of buffer from the filter paper during the run. The glass plates were placed on the edges of the opposing sides of the filter paper baths allowing the filter paper to extend closely down the inner side of the containers and into the buffer solution. Additional pressure on the plates may be obtained by adding 1 or 2 pounds of weight to the top of the plates after they are in place thus insuring a straight path of migration of the hemoglobin. The horizontal position of the glass plates was established with a spirit level and after a period of five minutes for equilibration, the siphon was removed or clamped off, and the current was applied. The preparative runs were made at 600 volts over a period of four to five hours. The paper was then removed from the plates and allowed to dry. Drying the paper before staining prevented the appearance of central pallor in a spot of high protein concentration. The dried paper was dropped into a cylinder of dye for 10 minutes, and then rinsed for several minutes in each of three successive acetic acid baths. After drying, the stained paper was passed through ammonia vapor to develop the blue color.

For a *quantitative* estimation of the protein pattern produced, the larger set of plates was used; this permitted use of larger volumes of hemoglobin solution in each spot and a longer run. The plates and paper were prepared as in the qualitative run, and 0.02 milliliter samples of a 4 per cent solution of carboxyhemoglobin were plated on the paper. The run was carried out at 600 volts over a period of 15 hours. Under these conditions, the migration of the spots from the baseline at the end of 15 hours varied from about 15 centimeters to 25 centimeters, depending upon the type of hemoglobin under examination. After removal of the paper from the plates and drying, samples to be analyzed with the *dye-elution* technique were stained as in the preparative runs, while samples to be analyzed with the *Folin method* were not stained. In either case, the papers were then placed on a medium sized paper cutter, and ¼-inch strips were cut from the paper parallel to the baseline, beginning at the point of maximum migration of hemoglobin and working back toward the baseline. These strips were numbered, and each in turn was cut perpendicular to the baseline, between the paths of migration of the hemoglobin samples. This provided a number of small pieces of filter paper, each representing a sample of known position from one of the carboxyhemoglobin specimens included in the run. With the *dye-elution technique* the pieces of paper were placed in separate tubes and the dye was eluted with 6 milliliter portions of 0.1 per cent sodium hydroxide and allowed to stand for one hour at room temperature. The optical density of the blue color was then read against a filter paper blank in a Coleman Universal spectrophotometer at 575 millimicrons. With the *Folin method* the protein was eluted from each piece of paper by allowing it to stand overnight in 2 ml. of 0.15 N sodium hydroxide. After elution, 1 ml. of 0.1 per cent copper sulfate and 6.0 ml. of 12.5 per cent sodium carbonate were added to the tubes. After one hour at room temperature, 1.0 ml. of Folin reagent, freshly diluted with twice its volume of distilled water, was added dropwise with shaking. After 15 minutes, the optical density of the blue color developed was read against a filter paper blank in a Coleman Universal spectrophotometer at 650 millimicrons.

The values obtained for the optical density using either the dye-elution technique or the Folin method may be plotted against the distance from the baseline and a curve obtained which resembles the configuration seen in moving boundary electrophoresis.

For many clinical purposes, a *semi-quantitative* method was adequate. This method was designed to provide information on the relative amounts of each component in a mixture of hemoglobins, expressed as a percentage of the total hemoglobin present. This information was obtained by using a 4 per cent solution of carboxyhemoglobin and employing the same technique used in the preparative run. The paper was stained and dried in the usual manner, and the separate components in the mixture were cut out, placed in individual tubes and the dye eluted with 6 milliliters of 0.1 per cent sodium hydroxide. The optical density of the solution is read at 575 millimicrons against a filter paper blank and the relative concentrations of the components established. Suitable known control samples of hemoglobin run with the unknown samples aid in accuracy of cutting out the spots.

RESULTS

Time of run. For preparative and semi-quantitative work, with the smaller plates, four or five hours were usually sufficient to establish the presence or absence of abnormal hemoglobin. The information so obtained was adequate for many clinical situations. For quantitative analyses, the greater migration of a 15-hour run permitted adequate sampling for the construction of an accurate elution curve. In certain instances, better separation of two hemoglobins was obtained with the longer than with the shorter run.

Voltage. Voltages up to 2,000 were used in various experiments. No significant advantage was noted using voltages in excess of 600, and 300 volts or less may be satisfactory for most purposes. The amperage at 600 volts was 2 to 4 milliamperes using the small plates and 7 to 9 milliamperes using the large plates. Veronal buffer of relatively low ionic strength, *i.e.*, 0.05, permitted operation at higher voltages without introducing a heating effect associated with increased amperage.

Buffer pH. Using relatively large volumes of buffer in the filter paper baths and placing the electrodes in separate containers tended to reduce pH changes during operation. The buffer was usually changed after three or four runs. Runs were made in cacodylate, phosphate and veronal buffers at pH values over a range of 6.5 to 9.2. It was possible to adjust the pH so that normal hemoglobin moved toward the positive pole and abnormal hemoglobin moved toward the negative pole, but no particular advantage was noted with this technique. Normal carboxyhemoglobin remained at the baseline at a pH of about 7.5. Runs made at a pH below 7.6 were less satisfactory due to increased trailing and smudging which produced less distinct spots. The most satisfactory buffer for both qualitative and quantitative analyses was veronal at pH 8.6.

Effect of volume and concentration of hemoglobin. Minor inaccuracies in pipetting or in concentration did not appear to affect the mobility. Volumes of hemoglobin solution greater than 0.02 and 0.03 milliliter caused considerable lateral spreading of the samples during the run. No attempt was made to use several layers of filter paper to increase the capacity of the system. In qualitative and semi-quantitative work, 0.005 milliliter samples were quite suitable, and for construction of an elution curve, 0.02 milliliter samples were used. Carboxyhemoglobin concentrations of 4 per cent gave neat appearing papers with well-defined protein spots, easily recognized before, as well as after, staining.

Effect of dialysis. Dialysis of the carboxyhemoglobin solutions at 4° C. against several changes of veronal buffer was carried out on a number of specimens. The most notable effect of prolonged dialysis was the elimination of a small amount of "head-trailing" present in some samples of undialyzed carboxyhemoglobin. Therefore, dialysis was considered to be unnecessary for most purposes. The final dilution of carboxyhemoglobin to the 4 per cent concentration used in the runs was carried out with veronal buffer of pH 8.6.

Effect of conversion of hemoglobin to carboxyhemoglobin. All hemoglobin samples were converted to the more stable carboxyhemoglobin before storage. This conversion did not appear to affect the mobility of the samples.

Effect of size of paper and plates. Using 0.005 milliliter samples, five separate hemoglobin preparations could be run simultaneously with the 8 by 30 centimeter glass plates. The larger plates are preferable for quantitative work because of a greater migration, larger volumes, increased sampling, and greater accuracy of elution curves. In addition, as many as eight or ten samples may be run simultaneously with the larger plates, and compared quantitatively after treatment under identical conditions.

Calibration curves. With the *Folin method,* increasing volumes of hemoglobin were added di-

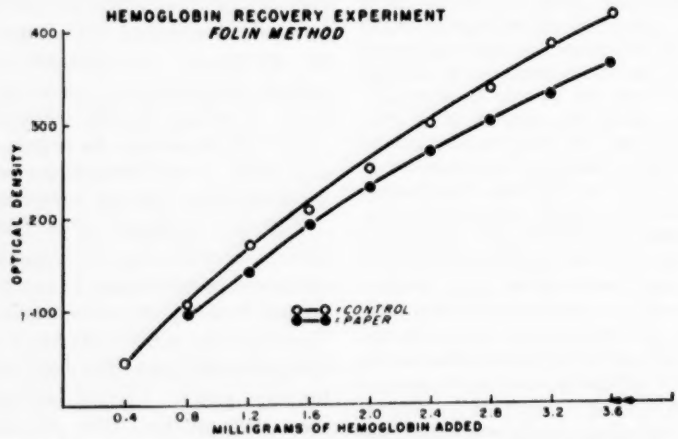

FIG. 1. CALIBRATION CURVE FOR THE MEASUREMENT OF HEMOGLOBIN USING THE METHOD OF FOLIN AND CIOCALTEU

TABLE I

Recovery experiments with artificial mixtures of normal and sickle hemoglobin—semi-quantitative technique, dye-elution method

Mixture of 90% normal and 10% sickle hemoglobin		Mixture of 75% normal and 25% sickle hemoglobin		Mixture of 50% normal and 50% sickle hemoglobin	
Normal	Sickle	Normal	Sickle	Normal	Sickle
Per cent of each hemoglobin recovered					
86	14	75	25	52	48
85	15	68	32	46	54
82	18	73	27	46	54
84	16	71	29	49	51
88	12	72	28	50	50
90	10	76	24	49	51
90	10	70	30	48	52
90	10	66	34	52	48
89	11	70	30	53	47
87	13	67	33	48	52
89	11	73	27	50	50
		70	30		
		67	33		

rectly to the tubes and these values plotted against the optical density. An essentially linear relation was obtained over the range of 0.100 to 0.500. Optimal amounts of hemoglobin for measurement with the dye-elution technique are of the order of 20 to 100 micrograms, and with the Folin method of the order of 0.3 to 1.5 milligrams. See Figure 1. For the *dye-elution* technique, increasing amounts of a hemoglobin solution were added to paper, and these values were plotted against the optical density of the eluted dye. An essentially straight line relation was obtained in the region of greatest sensitivity of the spectrophotometer at an optical density of 0.100 to 0.500.

Recovery experiments. With the *dye-elution* technique, known artificial mixtures of normal and sickle hemoglobin were run as in the semi-quantitative method and the relative concentrations determined. The data recorded in Table I indicate a recovery satisfactory for most clinical purposes. With the *Folin method,* values were compared for hemoglobin samples placed directly in the tubes with identical amounts placed on paper and then eluted with alkali. The lower curve in Figure 1 indicates approximately a 90 per cent recovery.

In Table II, figures obtained with the *semi-quantitative technique* are presented on the analysis of hemoglobin specimens from patients with sickle cell trait and sickle cell anemia. Among the patients with sickle cell trait, the values for sickle hemoglobin ranged from about 35 per cent to about 50 per cent. Among the patients with sickle cell anemia, the values for sickle cell hemoglobin were usually near 100 per cent, although one was as low as 72 per cent. It should be pointed out, however, that the mobility of fetal hemoglobin is intermediate between normal and sickle hemoglobin, and when fetal hemoglobin is mixed with either normal or sickle hemoglobin the fetal component cannot be readily distinguished by filter paper electrophoresis. For this reason, some of the values reported as sickle cell hemoglobin in patients with sickle cell anemia may be falsely high, due to admixture with unrecognized fetal hemoglobin.

Application of the quantitative technique. Elution curves were constructed for hemoglobins obtained from patients with a variety of anemias. In each case, the hemoglobin was run on the same piece of paper with a normal control. Hemoglobin obtained from patients with iron deficiency anemia, pernicious anemia, pernicious anemia with a 10 per cent reticulocyte response following the administration of Vitamin B_{12}, and congenital and acquired hemolytic anemia, all had elution curves identical with those of the normal control.

In Figures 2 and 3, elution curves are shown for five different hemoglobin patterns; all were obtained from a single sheet of paper in a single run. Patterns included are (1) normal, (2) sickle cell trait, (3) sickle cell anemia, (4) hemoglobin C

TABLE II

*Semi-quantitative analysis of hemoglobins from patients with sickle cell anemia and with sickle cell trait—dye-elution technique**

Sickle cell trait			Sickle cell anemia		
Patient	Normal hgb. %	Sickle hgb. %	Patient	Normal hgb. %	Sickle hgb. %
196	63	37	52	0	100
51	60	40	53	5	95
56	46	54	57	13	87
60	65	35	58	7	93
109	52	48	61	28	72
112	58	42	101	0	100
127	52	48	131	0	100
148	55	45	145	19	81
149	57	43	150	10	90
			152	0	100
			153	0	100
			176	0	100

* The values reported for "normal" hemoglobin may include some fetal hemoglobin. See text.

trait,[2] and (5) the naturally occurring mixture of hemoglobin C and sickle hemoglobin.

Application of the preparative and semi-quantitative methods. Filter paper electrophoresis of hemoglobin has been carried out on a number of patients with anemias of uncertain etiology, and in certain cases it has proved an extremely useful diagnostic aid. The presence of hemoglobin C, a new abnormal hemoglobin first described by Itano, and Neel (12) and by Kaplan, Zuelzer, and Neel (13), was readily demonstrated by filter paper electrophoresis. This hemoglobin (which was earlier called hemoglobin III or hemoglobin C) was detected in association with normal and with sickle hemoglobin, and in one case hemoglobin C was the only type of hemoglobin present. The clinical manifestations which are associated with the presence of the gene for hemoglobin C in the homozygous state and in the heterozygous states will be reported in a separate communication (14).

In addition to its usefulness in the detection of other abnormal hemoglobins, filter paper electrophoresis has been a valuable aid in distinguishing sickle cell trait from sickle cell anemia. While the clinical distinction between sickle cell trait and sickle cell anemia is usually readily made, occasionally patients with sickle cell anemia have their initial symptoms in adult life, or have symptoms of the homozygous sickling state without marked anemia. Other patients are seen who, although their erythrocytes are known to sickle, have other disease processes present which might account for the anemia observed. Although the velocity and morphologic characteristics of the sickling process frequently aid in distinguishing sickle cell trait from sickle cell anemia, it is sometimes difficult to make the distinction on the basis of the sickle cell preparation alone. The degree of reticulocytosis is often of value in distinguishing sickle cell trait from sickle cell anemia; however, patients with

[2] The nomenclature used for the varieties of human hemoglobin is that recommended by the Hematology Study Section of the Division of Research Grants of the National Institutes of Health. Blood, 1953, **8**, 386.

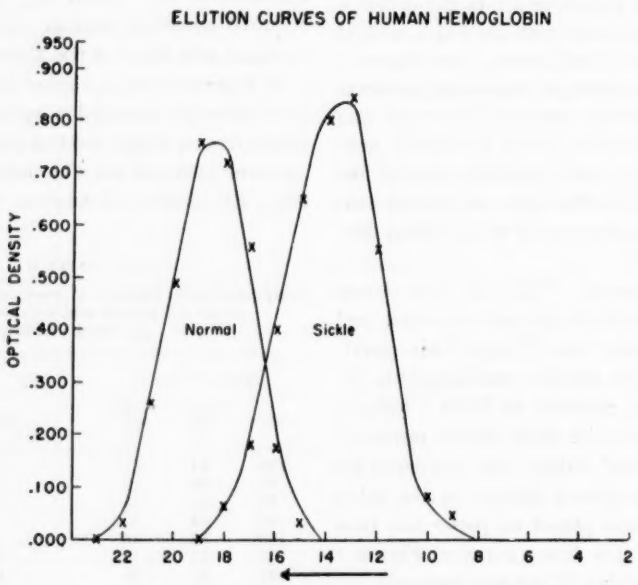

FIG. 2. DYE-ELUTION TECHNIQUE

Abscissa indicates centimeters of movement of the specimens of hemoglobin away from the baseline in the direction of right to left. The elution curves in Figures 2 and 3 were constructed from data obtained running the five species of hemoglobin simultaneously on a single sheet of paper. The separation of the curves into two figures is done only for clarity.

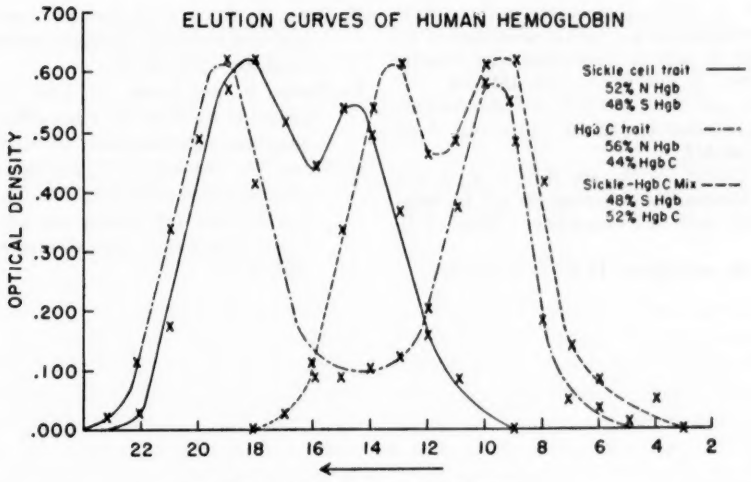

FIG. 3. DYE-ELUTION TECHNIQUE

See footnote to Figure 2.

sickle cell trait with recent blood loss may have an elevated reticulocyte count and, on the other hand, the reticulocyte count may be normal in certain patients whose hemoglobin is almost entirely of the sickle variety. It should be noted that the very rare double abnormal heterozygote for sickle hemoglobin and hemoglobin D(15) cannot be distinguished by electrophoresis, since all of the hemoglobin of such an individual would possess the mobility of sickle cell hemoglobin.

While filter paper electrophoresis is a rapid and simple method for the detection of normal adult hemoglobin, sickle hemoglobin and hemoglobin C, it is not satisfactory for the separation of fetal hemoglobin and sickle hemoglobin cannot be differentiated from hemoglobin D by this method. Therefore, filter paper electrophoresis should be combined with resistance to alkali denaturation (16, 17) for the estimation of fetal hemoglobin, and the solubility studies (15) for the determination of hemoglobin D.

SUMMARY

Methods are presented for the qualitative, semi-quantitative and quantitative analysis of human hemoglobin using filter paper electrophoresis. The effects of time of run, voltage, buffer pH, volume, concentration, dialysis, conversion to carboxyhemoglobin, size of paper and glass plates are discussed. Data on calibration curves and recovery experiments using the semi-quantitative and quantitative techniques are presented.

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CONCENTRATION GRADIENTS FOR INORGANIC I^{131} AND CHLORIDE IN MIXED HUMAN SALIVA [1]

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The ability of the thyroid gland to accumulate inorganic iodide and to maintain higher concentrations than those found in the serum has been described recently by Vanderlaan and Vanderlaan (1) and by Taurog, Chaikoff and Feller (2). This process is independent of organic iodination and can be dissociated from subsequent phases of the synthesis of thyroid hormone (3, 4).

The mechanism of the thyroidal "iodide trap" remains obscure. However, the existence of extrathyroidal sites for the selective concentration of inorganic iodide has been known for some time. In 1929, Lipschitz reported gastric juice/blood concentration ratios of 10 to 15 and saliva/blood ratios of 1.5 to 7.0 in Pavlov pouches and parotid fistulae of dogs which had been given up to twenty milligrams per kilogram of sodium iodide intravenously (5). After larger doses of iodide, gradients were reversibly depressed and the concentration of iodide in alimentary secretions progressively approached that of blood. Barkan and Leistner demonstrated that this transport mechanism was specific for inorganic iodide (6). Following the administration of an iodinated protein ("Iodtropon," *i.e.*, iodinated egg-white), they observed negligible amounts of iodide in gastric juice and saliva. Moreover, massive amounts of thyroxine did not increase alimentary concentrations of iodide beyond levels which might be anticipated from the metabolic degradation of thyroid hormone into inorganic iodide (7). The early German observations have been amply confirmed by later studies of the iodide content of saliva (8–12) and gastric juice (10, 13). However, analytical methods for stable iodide have differed widely and thereby complicated quantitative intercomparisons. Introduction of radioactive iodine obviated some of the analytical difficulties. Schiff, Stevens, Molle, Steinberg, Kumpe, and Stewart reported human gastric juice/serum gradients of I^{131} ranging from 1 to 70 and salivary/serum gradients from 7 to 700 (14). Their results are in substantial agreement with other studies employing I^{131} (15, 16, 17).

The quantitative agreement between thyroidal and salivary gradients for inorganic iodide as well as the phylogenetic and embryological similarities of these structures (18) prompted an investigation of the transport of I^{131} in mixed human saliva. Salivary concentration gradients for I^{131} have been related to simultaneously measured gradients for chloride and to 24-hour thyroidal uptakes of I^{131}. The effect of thiocyanate upon these functions has been evaluated.

METHODS

Subjects were convalescent hospital and ambulatory clinic patients, as well as normal laboratory personnel. All subjects were fasting and recumbent at the time of the observation and had abstained from smoking for at least the preceding one hour. Doses of 50 to 100 μc of carrier-free I^{131} dissolved in sterile saline were administered intravenously. Twenty to thirty minutes were allowed for equilibration. Blood specimens were collected at intervals of fifteen to thirty minutes during the following two hours. Sampling was not continued beyond the third hour since organically bound I^{131} may begin to appear at this time in the blood of patients with rapid rates of hormone manufacture (19). Collections of mixed saliva (*i.e.*, serous and mucous) were secured by standardized technique: subjects chewed $3 \times 1 \times 0.5$ cm. paraffin pledgets for five minutes and discarded all saliva collected during this period. For the next two minutes the mouth was rinsed continuously with tap water in order to remove any residual paraffin and saliva. A second five minute chewing period was then started with a fresh paraffin pledget and all of the saliva was collected in beakers. Specimens of saliva were transferred to graduated centrifuge tubes and their

[1] This investigation was supported in part by The Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. Da-49-007-MD-412.

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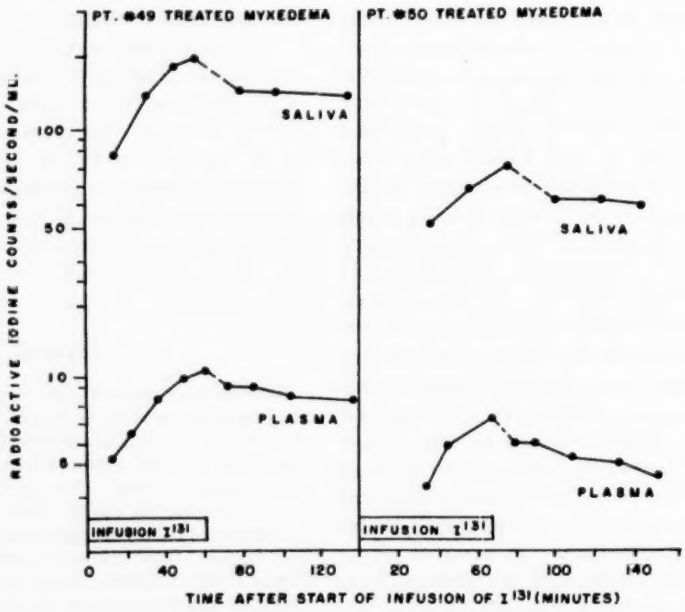
volumes recorded following centrifugation for fifteen minutes at 3000 r.p.m. Aliquots of serum and the centrifugated saliva were pipetted into weighed planchets and one drop of alkalinized one per cent gelatin supplemented with carrier iodide was added to each. Radioactivity was measured with a thin (1.4 to 1.8 mg. per cm.^{2}) mica-end window Geiger-Mueller counter. Dried planchets were counted sufficiently long to reduce the standard deviation of the count to less than one per cent. Geiger-Mueller tube characteristics and geometry were maintained constant. A correction for self-absorption was made in the radioactivity of each sample by means of a mass-absorption curve. Chloride analyses were performed in duplicate on similar aliquots of serum and saliva by the method of Volhard as modified by Van Slyke (20).

Specimens of saliva were chromatographed on strips of Whatman No. 2 filter paper in butanol and acetic acid (21). All of the radioactivity migrated as inorganic iodide.

Saliva/serum concentration gradients for I^{131} (S/P I^{131}) and chloride (S/P Cl) were calculated from the serum concentrations of these anions observed at the midpoint of the individual salivary collection period. Following the intravenous administration of I^{131}, blood levels of radioactivity are constantly declining. Therefore, the validity of concentration gradients for iodide determined from the simultaneous measurement of salivary and plasma I^{131} rests upon two assumptions. First, equilibration of iodide between plasma and salivary cells must be instantaneous, and second, the time lapse between the elaboration of saliva and its collection must be negligible. Theoretically, any delay in transport will result in errors in the estimation of the true concentration gradient, since radioiodide appearing in the saliva at any instant would have been extracted from the plasma at some earlier time. Thus, S/P ratios obtained during rising plasma concentrations would underestimate the true gradient, whereas a falling curve could result in overestimation. Artifactual overestimation would be maximal in subjects with hyperthyroidism, in whom the most rapid disappearance of I^{131} from the blood



S/P I^{131}	16.2	17.6	18.8	19.8	15.1	15.8	16.1	10.9	9.4	11.7	10.0	10.7	10.8
FLOW(ML/MIN.)	1.58	1.64	1.70	1.33	1.72	2.03	1.68	0.45	1.42	1.17	1.01	1.72	1.78

FIG. 1. ESTIMATION OF DELAY IN SALIVARY TRANSPORT OF I^{131} BY INFUSION TECHNIQUES

In Pt. No. 50, constancy of S/P I^{131} gradients during and following infusion of I^{131} denoted negligible delay in salivary transport. In Pt. No. 49, the rising gradients during the infusion indicated a finite "lag phase" which was shown by extrapolation to be approximately seven minutes.

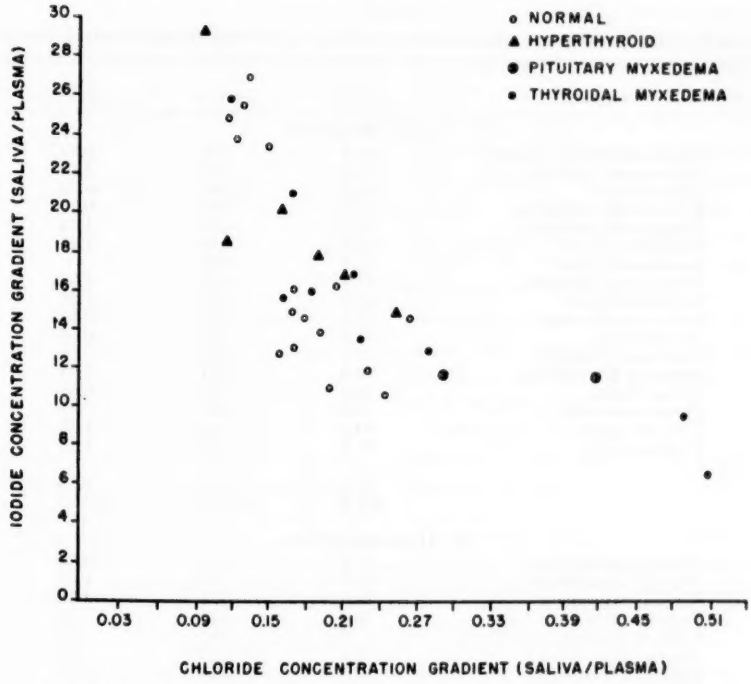


FIG. 2. THE RECIPROCAL RELATIONSHIP BETWEEN CONCENTRATION GRADIENTS FOR CHLORIDE AND IODIDE IN MIXED HUMAN SALIVA

occurs. The duration of the salivary "lag phase" may be estimated by extrapolating the simultaneous slopes of plasma and salivary radioactivity to their base line ordinates (in the case of a linear relationship) or by observing the relative positions of their peaks during ascent and descent. Such studies were performed in two athyreotic patients maintained on constant amounts of exogenous thyroid hormone. Rising plasma levels of I^{131} were produced by constant infusion; falling levels occurred after the infusion was discontinued. Specimens of plasma and saliva were collected during both periods by the methods which have been described previously. In subject No. 50, identical gradients were secured on the upslope as well as the downslope thereby indicating negligible delay in transport (Figure 1). In subject No. 49, the progressively increasing gradients during the linear infusion phase suggest a lag of approximately seven minutes (Figure 1). Correction for this delay yielded a constant S/P ratio of 20 throughout the period of rising plasma radioactivity. The depressed gradients during the post-infusion phase cannot readily be explained. Despite the crudeness of these measurements and obvious over-simplification of complex kinetics, the results are consistent with an extremely rapid, iodide transport between serum and saliva. Hence, in all subsequent studies, no correction was made for error due to delay in equilibration and/or "dead space."

Six subjects were given 1.5 to 3 grams of sodium thiocyanate intravenously. Delivery was made by a single injection over a four-minute period. Saliva/serum gradients for I^{131} and Cl were obtained prior to and following the administration of thiocyanate. The possible interference of thiocyanate in the estimation of chlorides was examined by *in vitro* recovery experiments. Addition of 0.1 to 0.5 milli-equivalents of thiocyanate to one milliliter specimens of serum or saliva containing known amounts of chloride did not interfere with chloride estimation by more than two per cent, presumably as a result of destruction of the thiocyanate during digestion of the proteins.

RESULTS

Observations were made in thirty-five individuals. All subjects who were receiving any medication which might influence thyroidal or salivary function were excluded from the present series.

The rates of disappearance of radioactivity from saliva and blood were approximately equal after the intravenous administration of I^{131}. Serial estimates of saliva/serum gradients (S/P ratios) for I^{131} and Cl were averaged for the compilation of Figure 2 and Table I. In the entire series, the

TABLE I

Metabolic activity and simultaneous concentration gradients for iodide and chloride in mixed human saliva

Subject file No.	Diagnosis	S/P I^{131}	S/P Cl	Flow (*ml./min.*)	24-hour thyroidal uptake of I^{131}
	I. Normal Metabolism				
7	Rheumatic Heart Disease	23.5	.150	0.85	33
9	Rheumatoid Arthritis	24.9	.119	2.53	12
18	Neurasthenia	16.2	.204	0.73	—
23	Rheumatoid Arthritis	27.0	.144	0.74	21
24	Rheumatoid Arthritis	14.6	.264	1.16	—
25	Nephrocalcinosis	14.6	.179	1.15	21
26	Esophageal Ulcer	21.1	.170	—	13
28	Rheumatoid Arthritis	11.9	.229	4.70	12
31	Nephrocalcinosis	13.1	.171	2.34	24
34	Laboratory Worker	25.5	.129	0.91	17
35	Neurasthenia	17.1	.160	0.65	14
36	Infectious Hepatitis	12.7	.160	—	17
39	Subacute Thyroiditis	10.9	.201	1.94	0
42	Neurasthenia	14.9	.170	0.65	18
43	Thrombophlebitis	13.8	.191	2.11	—
44	Varicose veins	10.6	.243	1.55	—
45	Pyelonephritis	23.8	.124	1.15	21
Mean		17.4	.177	1.54	
S.E.M.*		±1.4	±.010	±0.27	
	II. Hypometabolism				
3	Panhypopituitarism	11.6	.414	0.91	0
8	Surgical Myxedema†	16.8	.217	1.70	4
10	Surgical Myxedema†	13.6	.224	2.05	5
11	Surgical Myxedema†	25.8	.120	1.30	3
13	Primary Myxedema	15.7	.188	1.63	1
14	Surgical Myxedema‡	16.0	.183	1.76	0
20	Surgical Myxedema‡	12.9	.278	1.93	12
22	Panhypopituitarism	11.7	.291	1.93	2
33	Surgical Myxedema†	6.6	.506	1.46	2
38	Surgical Myxedema†	9.6	.484	0.79	4
Mean		14.0	.291	1.55	
S.E.M.		±1.6	±.042	±0.14	
	III. Hypermetabolism				
1	Thyrotoxicosis	29.4	.099	1.23	96
2	Thyrotoxicosis	20.5	.160	1.23	72
6	Thyrotoxicosis	14.8	.251	2.05	53
11	Thyrotoxicosis	16.8	.212	1.30	72
29	Thyrotoxicosis	18.9	.178	0.79	71
32	Thyrotoxicosis	18.4	.116	2.65	76
Mean		19.8	.169	1.54	
S.E.M.		±2.1	±.023	±0.28	

* Standard Error of the Mean.
† Post-operative: total thyroidectomy for carcinoma.
‡ Post-operative: subtotal thyroidectomy for hyperthyroidism.

mean S/P I^{131} was 16.8 ± 0.98;[3] S/P chloride averaged $0.210 \pm .017$[3] (Table I). Salivary chloride content never equalled that of serum. When concentration gradients for iodide were plotted against those which had been simultaneously determined for chloride, an inverse curvilinear relationship of high statistical significance was observed (r, − 0.74; p, less than 0.001 [22]) (Figure 2). Even greater correlation was obtained when log S/P I^{131} was plotted versus log S/P Cl (r, − 0.87).

During the test periods, mixed saliva was secreted at an average rate of 1.54 ± 0.15 ml. per min.[3] No consistent relationship was noted between rates of flow and electrolyte composition (Table I). Mixed saliva could not be collected with an accuracy comparable to that obtained in the measurement of the concentration of I^{131} and chlo-

[3] Mean ± standard error of the mean.

TABLE II

Constancy of salivary gradients

Subject file No.	Interval	S/P I^{131}	S/P Cl	Flow (*ml./min.*)
1	—	29.4	.099	1.23
	11 weeks	29.3	.117	1.16
25	—	14.6	.179	1.15
	3 weeks	12.6	.249	2.30
31	—	12.6	.111	2.20
	4 weeks	13.1	.171	2.34

ride in aliquots. Since a salivary mechanism for reabsorption of water has never been demonstrated, it is doubtful whether measurements of clearance utilizing flow values offer any more reliable estimate of osmotic work than S/P ratios.

Reproducibility of paraffin stimulation was assessed in three individuals by repeating studies at widely spaced intervals. Constancy of S/P I^{131} ratios within the same individual was seen (Table II).

Intravenous administration of 1.5 to 3.0 grams of sodium thiocyanate caused a sixty to eighty per cent reduction in salivary gradients for I^{131} (Table III). In no instance was the concentrating mechanism abolished completely; the lowest observed S/P I^{131} was 2.8. Comparable alterations in flow or chloride transport were not seen (Table III). Depression of I^{131} transport could be demonstrated within two minutes after administration of thiocyanate. In some subjects this reduction persisted at constant levels for sixty to ninety minutes (Figure 3). Prior treatment with sixty mg. of Thyrotropin[4] for five days did not appreciably modify the effectiveness of thiocyanate in subject No. 3. The promptness of thiocyanate action was viewed as additional evidence for rapid transport across salivary cells.

Correlation between the magnitude of the S/P ratios for I^{131} and the 24 hour uptake of I^{131} by the thyroid could not be demonstrated. Since salivary

[4] Thyrotropin, Armour, Lot K46808R was kindly supplied by Dr. C. J. O'Donovan of Armour Laboratories. Dosages are cited in mg. equivalent to Armour standard (2R3). Potency was approximately 0.5 U.S.P. units per mg. equivalent.

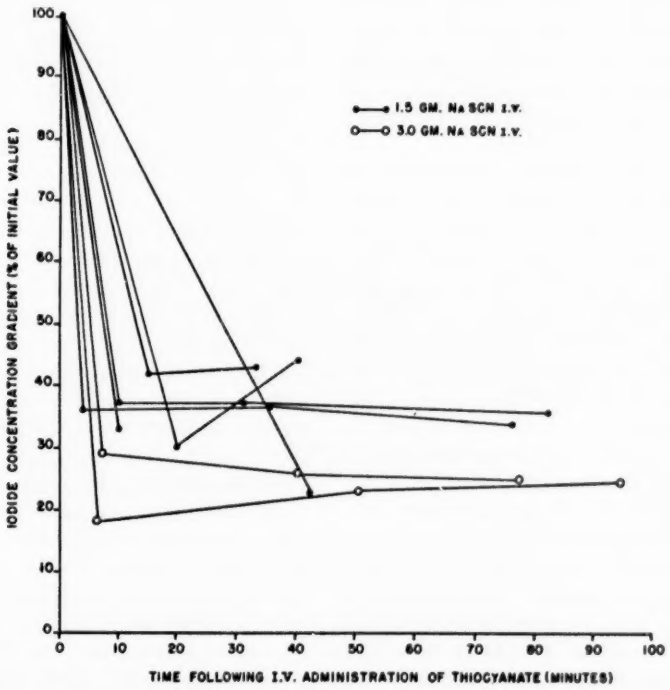


FIG. 3. DEPRESSION OF SALIVARY SECRETION OF IODIDE BY THIOCYANATE ION

TABLE III

Effect of thiocyanate upon salivary transport mechanisms

Subject file No.	Dose	S/P I^{131}		S/P Cl		Flow		Salivary Cl	
		Pre†	Post†	Pre	Post	Pre	Post	Pre	Post
	Gm.					*ml./min.*		*mEq./L.*	
3	1.5	11.6	4.1	.414	.386	0.91	0.95	42.9	40.3
3a*	1.5	8.1	3.4	.585	.565	1.47	1.12	60.7	57.2
13	1.5	15.7	5.8	.188	.197	1.63	1.37	18.2	18.9
14	1.5	16.0	3.7	.183	.202	1.76	2.18	18.1	20.0
31	1.5	13.1	4.6	.171	.143	2.34	2.08	18.8	15.7
25	1.5	12.6	4.6	.249	.239	2.30	2.16	27.6	26.4
43	3.0	10.6	2.8	.243	.297	1.55	1.66	23.8	29.1
44	3.0	13.8	3.0	.191	.233	2.11	1.93	18.4	22.2

* Subject was given 30 units of Thyrotropin, Armour, during the five days preceding the study.
† Pre and post refer to average values preceding and following the administration of thiocyanate.

gradients for iodide were maintained in two patients with untreated panhypopituitarism, in one subject with primary thyroid failure, and in seven subjects with myxedema following surgical thyroidectomy, it may be inferred that concentrating ability is operative even when production of either thyrotropin or thyroid hormone is subnormal.

DISCUSSION

Elaboration of any secretion which differs in composition from that of an ultrafiltrate of plasma entails the performance of osmotic work. Early studies of the metabolic concomitants of salivary activity disclosed that such work is accompanied by the extraction of glucose and oxygen from the perfusing blood and the addition of lactic acid, and phosphates to the venous effluent (23). Simultaneous depletion of glandular glycogen and creatine phosphate has also been described (24). Some correlation between levels of metabolism and osmotic efficiency might be expected. It is interesting, therefore, that the three individuals (subject Nos. 3, 33, and 38) who were least able to concentrate iodide or to withhold chloride were in the hypometabolic group. The remaining subjects exhibited no simple correlation between osmotic performance and metabolic activity.

An inverse relationship between salivary chloride and iodide has been demonstrated in the present studies, despite the marked disparity in their concentrations in the plasma. Such reciprocity need not denote a common mechanism for transport. In parotid saliva, chloride concentration rises with increasing rates of flow (25). It has been suggested that iodide concentration in mixed saliva increases with decreasing rates of flow (17). Therefore, the correlation between S/P ratios for chloride and I^{131} may reflect merely an inverse dependency upon flow. Even with standardized stimulus and technique of collection, this could not be demonstrated in our heterogeneous population. It is difficult to evaluate the contribution of flow to the ionic composition of mixed saliva. Concentration of iodide occurs largely in the serous component (17). Thus, admixture of varying quantities of mucus might not distort the reciprocity between concentration gradients, but might obscure their dependency upon rates of serous secretion.

Thyroidal (1), gastric (26) and renal (27) transport mechanisms may be affected by thiocyanate. In the present studies, a salivary locus of thiocyanate action has also been demonstrated. In some subjects concentration gradients for I^{131} in saliva remained constantly depressed for two to three hours following intravenous administration of thiocyanate despite the fact that levels of thiocyanate in the plasma must have undergone fivefold reduction during this interval, *i.e.*, from an initial dilution within the blood volume alone to ultimate equilibration throughout the "thiocyanate space" (28). The reduction in concentration of iodide in saliva was unaccompanied by comparable changes in the exclusion of chloride from saliva. Thus, thiocyanate produced a dissociation of the reciprocity between chloride and iodide. This would argue against a common salivary transport for chloride and iodide although the possibility cannot be excluded that there exists a multi-stage system of transport which is shared at a point not affected by thiocyanate.

To what extent salivary and thyroidal mechanisms for the accumulation of inorganic iodide are identical remains unanswered. Certainly absolute concentration gradients and extraction ratios are similar in magnitude at both sites. Furthermore, blockade by thiocyanate is equally effective in the thyroid and salivary glands. However, actual concentration of thiocyanate occurs in saliva (29) whereas there is no evidence for such a phenomenon within the thyroid (30, 31). Moreover, a well-defined relationship between thyroidal activity and salivary gradients cannot be demonstrated, although in some instances of profound hypometabolism, the osmotic efficiency of salivary glands is depressed.

SUMMARY

1. In thirty-five human subjects, salivary concentration gradients for I^{131} have been correlated with thyroidal activity and with simultaneous measurements of gradients for chloride. Mixed saliva was collected following standardized stimulation with paraffin; reproducibility of the technique was assessed by serial observations at intervals varying from minutes to weeks.

2. Infusion techniques failed to reveal any significant "lag phase" between cellular penetration and salivary elaboration of I^{131}. Saliva/serum gradients (S/P ratios) were therefore derived by concurrent estimates of plasma and salivary concentrations of chloride and I^{131}. S/P I^{131} averaged 16.8 ± 0.98; S/P chloride averaged $0.210 \pm .017$. Osmotic performance did not differ among euthyroid and hyperthyroid individuals. Three subjects with profound hypometabolism, showed diminished capacity to concentrate iodide and to withhold chloride.

3. Administration of thiocyanate produced a sixty to eighty per cent reduction in S/P I^{131} within two to six minutes, without systematic alterations in salivary flow or chloride.

4. Curvilinear reciprocity between S/P ratios for iodide and chloride could be demonstrated. The evidence is inadequate to assess whether this represented interaction of anionic transport mechanisms or merely an inverse dependency upon flow.

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THE DEMONSTRATION OF TYPE SPECIFIC STREPTOCOCCAL ANTIBODY BY A HEMAGGLUTINATION TECHNIQUE EMPLOYING TANNIC ACID[1]

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Immunity to the group A streptococcus has been shown to be associated with the formation of antibody against the type-specific component of the streptococcus, the M protein of Lancefield (1, 2). The techniques that have been employed in studies of this antibody include agglutination (3), precipitation (4), mouse protection (5), and bacteriostasis (6, 7). For practical purposes, in the study of human infections, all of these methods except the bacteriostatic test have proved to be unsatisfactory. The latter test, as described by Rothbard (7), yields reliable results but the numerous technical difficulties inherent in the procedure have made it desirable to find another method for the demonstration of type specific antibodies.

The presence of various antibodies has been demonstrated by the adsorption of antigenic components of bacteria to the surface of erythrocytes, rendering the cells agglutinable by specific antisera. The widest application of this technique has been in the demonstration of antibodies to a component of the tubercle bacillus (8, 9); the technique has also been applied in serological studies of H. influenzal meningitis (10), histoplasmosis (11), glanders (12), scrub typhus (13), mumps (14), tularemia (15), streptococcal infections and rheumatic fever (16), and infections with *E. coli* (17).

In this laboratory, repeated attempts were made to adsorb M protein to erythrocytes and then demonstrate their agglutination by specific antibody. No adsorption could be demonstrated, even when erythrocytes from sheep, rabbit, and man were exposed to high concentrations of the antigen for prolonged periods. Other methods were then sought which would modify the cell surface and facilitate the adsorption of M protein. Peck and Thomas (18), in a study of the capacity of tannic acid to render erythrocytes susceptible to hemolysis by human complement, demonstrated that tannic acid has an apparent affinity for both red cells and complement, and when adsorbed on the cell surface is capable of bringing complement, or a component of complement, into close relation with the cell surface. The concentration of saline used as a diluent was of major importance, 0.7 per cent saline proved to be optimal. Human red cells treated with tannic acid were not susceptible to lysis by sera from the rabbit, guinea pig, mouse, or sheep.

Boyden (19), by treating sheep erythrocytes with tannic acid prior to adsorbing P.P.D. antigen to their surfaces, was able to detect antibodies in sera from tuberculous patients in dilutions up to 100-fold greater than those shown by the direct sensitization technique of Middlebrook and Dubos (8). The results of the two methods, however, did not always correlate and the author suggests that more than one antibody may have been involved. By the same technique ovalbumin, horse serum globulin and albumin, chicken globulin, and a streptococcus protein preparation were adsorbed to sheep red cells, and the cells were agglutinated by the corresponding antibodies.

The present paper is concerned with the demonstration of type-specific antibodies against the M protein of group A streptococci, by a hemagglutination reaction involving human erythrocytes treated with tannic acid prior to the adsorption of M antigen.

MATERIALS AND METHODS

Human erythrocytes. Human group O blood was collected aseptically in equal volumes of Alsever's solution

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(20) and stored at 4° C. The erythrocytes so stored could be used for several weeks. At the beginning of each day an aliquot of cells was washed three times in normal saline and was then ready for use. Satisfactory results were also obtained with group O blood-bank cells which had been collected within the preceding several days. When blood bank cells were used they were collected by centrifugation and added to equal volumes of Alsever's solution.

Saline solution. Saline solution was used in a concentration of 0.75 per cent in all parts of the procedure except in the initial washing of the erythrocytes. The advantages of this concentration of saline over normal saline were that nonspecific agglutination occurred less frequently, and the agglutination pattern of the erythrocytes was easier to read.

Tannic acid. Fresh tannic acid solutions were prepared daily using 0.75 per cent saline as the diluent. Mallinckrodt tannic acid (analytical reagent, lot number 1764) was used throughout these experiments.

M protein. M protein of group A streptococci was prepared by the acid hydrolysis method of Swift, Wilson, and Lancefield (21), and the group specific C polysaccharide was removed by several extractions with 95 per cent ethyl alcohol (22). The final product was suspended in 8 cc. of normal saline per liter of original culture and was designated as "undiluted" M protein. Various dilutions of this material were prepared in 0.75 per cent saline. These preparations consist of only partially purified M protein but for convenience will be referred to in this publication as "M protein." It should also be emphasized that M protein is a mixture of heterogeneous proteins, and there is no available method for determining whether various antigenic properties of this material are due to the activity of a single substance or a multitude of substances.

Sera. Type-specific antistreptococcus rabbit sera were obtained through the courtesy of the Public Health Service Communicable Disease Center Streptococcus Laboratory, Chamblee, Georgia, and sera from patients with streptococcal infections were provided by the Streptococcal Disease Laboratory, Warren Air Force Base, Wyoming. All sera were heated at 56° C. for 30 minutes before use.

Complement. The complement used was lyophilized guinea pig complement (Lyo Complement, Sharp and Dohme). Dilutions of complement were made with 0.75 per cent saline solution.

Sensitization of erythrocytes with tannic acid. One volume of tannic acid solution was added to two volumes of 1 per cent erythrocyte suspension and allowed to stand at room temperature for 30 minutes. The treated cells were then washed three times with 0.75 per cent saline solution.

Adsorption of M protein to erythrocytes treated with tannic acid. One part of tannic acid treated erythrocytes was added to fifty parts of the desired dilution of M protein and the mixture placed in a water bath at 37° C. for one hour, shaking sufficiently to resuspend the cells after the first 30 minutes. The cells were washed three times with a 0.75 per cent saline solution and then diluted to make a 2 per cent suspension. The final dilution in most experiments was made in 0.75 per cent saline; in some it was made in a 1:100 dilution of normal rabbit serum in 0.75 per cent saline. These erythrocyte suspensions were used in the final test and were satisfactory for use only on the day of preparation.

Hemagglutination test. Chromic acid-cleansed 100 × 13 mm. test tubes were used in all tests. Serial dilutions of sera were made in 0.75 per cent saline or, in some of the experiments to be described, in a 1:100 dilution of normal rabbit serum in 0.75 per cent saline. To 0.2 cc. amounts of each serum dilution was added 0.2 cc. of the erythrocyte suspension. The tubes were incubated in the 37° C. water bath for two hours, shaking at 30 minute intervals, and then placed at 4° C. until the following morning. After 30 minutes at room temperature, readings were made by the pattern of the red cells on the bottom of the tube. When agglutination occurred, the cells settled out in a wide area, with varying degrees of clumping. In negative tests, the cells formed a small button in the tip of the tube. Readings could not be made by agitation of the tubes and observation of suspended clumps of agglutinated erythrocytes, since even those showing maximal agglutination were readily dispersed by shaking. The titer of agglutination was considered to be the highest dilution of serum showing definite clumping, and was designated by the dilution of serum before mixture with the erythrocytes.

Controls were run with all tests and included erythrocytes treated with tannic acid and M protein with no serum present, and erythrocytes treated with tannic acid alone.

Hemolytic test. The hemagglutination test could, under certain conditions described below, be converted into one involving hemolysis instead of agglutination. This was accomplished by adding to each tube in the agglutination test, after overnight refrigeration, 0.2 cc. of a 1:5 dilution of guinea pig complement. Readings for hemolysis were made after 1 hour in the 37° C. water bath.

EXPERIMENTAL RESULTS

The optimal concentration of tannic acid. Since hemagglutination is known to occur in the presence of tannic acid alone, it was first necessary to determine the concentrations of this material which could be used without causing agglutination. To do this, various concentrations of tannic acid were added to a 1 per cent solution of washed red cells, in the proportion of one volume of tannic acid to two of cell suspension. The mixture was allowed to stand at room temperature for 30 minutes, and the cells washed three times. The erythrocytes were then resuspended in 0.75 per cent saline to make a 1 per cent suspension, placed in the refrigerator overnight, and read for agglutination. Definite agglutination occurred in cell suspensions which had been exposed to tannic acid in concen-

trations higher than 0.004 per cent; the concentration of 0.004 per cent caused questionable agglutination; concentrations of less than 0.004 per cent caused no agglutination. On the basis of this observation a concentration of 0.001 per cent was arbitrarily selected for use in all the subsequent experiments to be described.

The optimal concentration of M protein. Cells which had been treated with tannic acid were found to be rapidly agglutinated by the addition of M protein, when sufficient concentrations of the protein were used. Untreated erythrocytes were not agglutinated by the same or higher concentrations. It was therefore necessary to titrate M protein in the presence of treated cells in order to find a concentration in which interference by this type of agglutination did not occur.

Dilutions of M protein were added to erythrocytes treated with 0.001 per cent tannic acid; the mixtures were incubated at 37° C. for one hour, and the red cells washed three times and resuspended in 0.75 per cent saline to make a 1 per cent suspension. A reading for agglutination was made after overnight refrigeration. Agglutination of tannic acid treated cells occurred with all M protein preparations in dilutions of 1 : 25 or less and with some preparations in dilutions as high as 1 : 200, different titers being found not only with M protein from different types of streptococci but also with different batches of M protein from the same type of streptococcus. In view of the differences between the preparations, it was evident that a similar titration of M protein would be necessary in all subsequent experiments in order to avoid spontaneous agglutination but still utilize the maximal concentration of antigen for adsorption to erythrocytes.

Because of the possibility that the agglutination observed might be a specific property of M protein, attempts were made to block it by incubating type-specific rabbit sera with the M protein before the addition of the treated erythrocytes. Serial dilutions in amounts of 0.25 cc. were made of type 1, 14, and 24 rabbit antistreptococcus sera as well as normal human sera, and to these tubes was added 0.25 cc. of a 1 : 4 dilution of type 1 M protein. After incubation at 37° C. for one hour, 0.5 cc. of a 2 per cent suspension of tannic acid-treated erythrocytes was added to each tube. Following two hours in the 37° C. water bath and overnight refrigeration, readings were made for agglutination of the red cells. It was found that inhibition of agglutination was caused by all of the sera employed in dilutions up to 1 : 40 or 1 : 80. The observations provided no evidence that the agglutination by low dilutions of M protein of tannic acid treated cells is due to the type-specific antigen; the inhibition by unrelated sera suggests that it may represent a nonspecific property of the preparations. Similar hemagglutination was produced by a much more highly purified preparation of type 1 M protein (23), obtained through the courtesy of Dr. Rebecca C. Lancefield.

Type-specific hemagglutination of erythrocytes treated with optimal amounts of tannic acid and M protein. The above experiments showed that the preparation of erythrocytes which were not spontaneously agglutinated by tannic acid or M protein, necessitated the use of tannic acid in a concentration of 0.001 per cent and M protein in a concentration that depended on the agglutinating property of individual preparations. If nonspecific hemagglutination was avoided by these measures, specific agglutination of cells treated with tannic

TABLE I

Agglutination by antistreptococcus rabbit sera of human erythrocytes treated with tannic acid and different concentrations of M protein

Antistreptococcus serum type*	Type 24 M protein dilution†						
	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096
24	+‡	+	+	+	+	+	−
1	−	−	−	−	−	−	−
Control, no serum	−	−	−	−	−	−	−

* A 1:100 dilution of the indicated serum was used in all tubes.

† Erythrocytes were treated with 0.001 per cent tannic acid and the indicated dilution of M protein before being exposed to the serum dilutions.

‡ + means agglutination of the erythrocytes; − means no agglutination.

acid and M protein could be demonstrated by antistreptococcal rabbit serum, as is shown by the following data.

An experiment which illustrates the activity of various amounts of M protein in the hemagglutination test is shown in Table I. Two-fold dilutions of a type 24 M preparation were used for adsorption on the tannic acid-treated cells, as described in the section on Materials and Methods. A 1 : 100 dilution of type 24 rabbit antistreptococcus serum was mixed with cells exposed to each dilution of M protein. Hemagglutination occurred in all tubes through the dilution of 1 : 2048. The specificity of the reaction is indicated by the absence of hemagglutination in control tubes containing type 1 serum or saline. It was evident from the experiment that adsorption of M protein to the treated cells had occurred to an extent sufficient to demonstrate the presence of small quantities of the antigen.

The hemagglutination test with various types of M protein and rabbit antisera. A cross titration of 7 different serological types of rabbit antisera was carried out in the presence of cells which had been prepared with corresponding types of M protein. In each instance the cells were exposed to a 1 : 250 dilution of M protein. The results indicate that hemagglutination occurred in high titer with each mixture of serum and homologous M protein (Table II).

Cross-reactions with other types of antisera were observed in the cells treated with type 3 M protein, although the titers obtained with heterologous sera were considerably lower than those with homologous sera. One batch of type 19 rabbit antistreptococcus serum has been observed to cause cross agglutination in high titer with many heterologous types of M protein.

Figure 1 shows a photograph of a typical test, using type 24 M protein-treated erythrocytes and types 1, 5, 14, and 24 rabbit sera. The bottom line of tubes represents a positive reaction with the type 24 serum, beginning with a 1 : 100 dilution and showing an endpoint at 1 : 12,800. The other rows show the typical appearance of negative reactions.

The hemolytic effect of guinea pig complement on treated cells in the presence of specific antiserum. It has been shown that tannic acid-treated human erythrocytes are hemolyzed by human complement, but not by guinea pig complement (18). Accordingly, attempts were made to determine if guinea pig complement, when added to the regular test, would change it to one that could be read by hemolysis instead of hemagglutination. Guinea pig complement was added to the tubes containing the mixtures of M-sensitized cells and antiserum, after the latter had been allowed to stand overnight at 4° C. After the addition of complement the cells were incubated at 37° C. for one hour and then read for hemolysis. When preparations of type 24 M protein were used, hemolysis occurred in significant titers. Little or no hemolysis occurred, however, even when the hemagglutinating titers were high, when types 1, 3, 5, and 14 M protein and antisera were used. The sensitivity of the hemolytic test using type 24 M protein varied with different batches of the antigen but in all instances showed higher titers than were seen with preparations made from other types of streptococci. The hemolysis seemed to be specific in that none occurred in the absence of specific hemagglutination.

The specific inhibition of hemagglutination by

TABLE II

Titration of antistreptococcus rabbit sera with erythrocytes treated with tannic acid and various types of M protein

M protein type*	Type antistreptococcus rabbit sera						
	1	3	5	12	14	17	24
1	1:2,560†	—	—	—	—	—	—
3	1:80	1:10,240	1:160	1:160	—	—	1:640
5	—	—	1:5,120	—	—	—	—
12	—	—	—	1:2,560	—	—	—
14	—	—	—	—	1:1,280	—	—
17	—	—	—	—	—	1:5,120	—
24	—	—	—	—	—	—	1:2,560

* Erythrocytes treated with 0.001 per cent tannic acid before being exposed to a 1:250 dilution of the indicated M protein.

† The figures are the highest dilution of serum that agglutinated the red cells. A negative reaction indicates that there was no hemagglutination at a 1:80 dilution or higher.

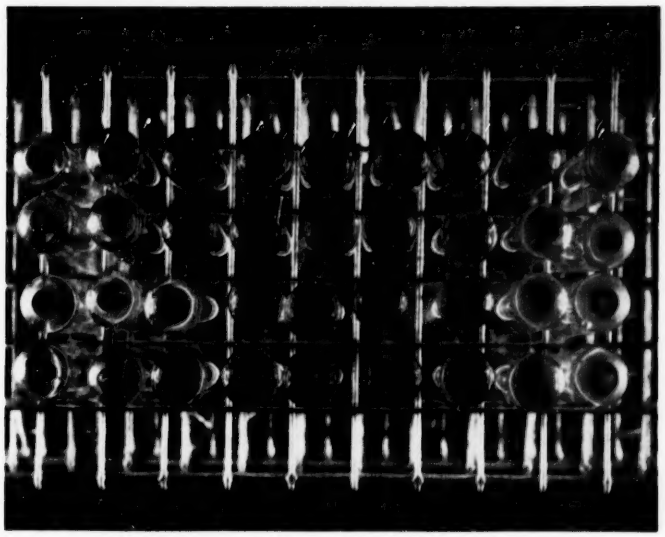

FIG. 1. THE HEMAGGLUTINATION TEST USING RABBIT ANTISTREPTOCOCCUS SERA AND ERYTHROCYTES TREATED WITH TANNIC ACID AND TYPE 24 M PROTEIN

The bottom row of tubes shows a positive reaction with type 24 serum, beginning with a 1 : 100 dilution. The other rows show negative reactions with types 1, 5, and 14 sera.

homologous M protein. In order to study further the degree of specificity of the hemagglutination reaction with erythrocytes treated with tannic acid and M protein, the effect of the addition of homologous and heterologous M protein to the type specific serum before mixing the latter with M protein treated erythrocytes was investigated. The serum was serially diluted in the usual manner, and the erythrocytes were prepared with tannic acid and homologous M protein. Next, 0.2 cc. amounts of a 1 : 100 dilution of M protein were added to the tubes containing the serum dilutions, and the tubes incubated at 37° C. for one hour. The erythrocytes were then added in the usual manner. When the added M protein was of the same type as the anti-M antibody in the serum, agglutination was inhibited. When the M protein of a heterologous type was used, agglutination was not inhibited; in fact, there sometimes occurred a slight enhancement of agglutination in these tubes. Table III illustrates the results of an experiment showing the blocking effect of adding type 14 M protein to homologous serum before mixing with tannic acid treated erythrocytes.

The hemagglutination test with acute and convalescent sera from patients with streptococcal infections. After it had been demonstrated that rabbit antistreptococcus sera would specifically agglutinate erythrocytes treated with tannic acid and M protein, attempts were made to determine if specific antibodies could be demonstrated in the sera of patients convalescent from Group A streptococcal infections. Acute and convalescent sera were obtained from patients with streptococcal upper respiratory infections; typeable group A streptococci had been isolated in large numbers in cultures of the throats of these patients, and type-specific bactericidal antibodies had been demonstrated in the convalescent sera of all. The sera were tested with erythrocytes sensitized with homologous M protein, and also with control cells prepared with heterologous M protein. The results of one such experiment using sera from patients with type 24 streptococcal infections and erythrocytes treated with types 24 and 14 M protein are shown in Table IV. In five of the six patients there was a two-fold or greater increase in the agglutination titer of the convalescent serum

TABLE III

The effect of homologous and heterologous M protein on the agglutination by type 14 rabbit serum of type 14 M protein-treated erythrocytes

Type of M protein added*	Dilution of type 14 rabbit serum†					
	1:100	1:200	1:400	1:800	1:1,600	1:3,200
14	—	—	—	—	—	—
1	+	+	+	+	+	—
5	+	+	+	+	+	—
24	+	+	+	+	+	—
none, control	+	+	+	+	—	—

* A 1:100 dilution of the indicated M protein was added to each serum dilution, and these incubated for one hour before adding the treated erythrocytes.

† Erythrocytes were treated with 0.001 per cent tannic acid and a 1:250 dilution of type 14 M protein before being exposed to the indicated serum dilutions.

for the homologous antigen. No hemagglutination occurred with either the acute-phase or convalescent serum of one patient.

Table IV also shows that reactions of the same pairs of sera occurred with erythrocytes treated with type 14 M protein. Hemagglutination was noted in four of the six pairs of sera; there was an increase in titer with one pair of sera while the other three showed the same titers in the acute-phase and convalescent sera.

Acute and convalescent phase sera from patients with infections due to types 1, 5, 14, and 24 streptococci have also been tested for the presence of hemagglutinating antibodies. Significant increases have been demonstrated in the convalescent sera in the majority of cases with the homologous type of M protein. In addition, however, nonspecific cross reactions similar to those described above were encountered in a high percentage of cases.

In most instances there was direct correlation between the presence of type-specific bactericidal antibodies and type-specific hemagglutinating antibodies. In several cases, however, hemagglutination did not occur with sera known to contain bactericidal antibody, suggesting the possibility that the antibodies responsible for the two reactions may not be identical.

The blocking effect of heterologous M preparations on nonspecific cross-reactions. The occurrence of nonspecific cross-reactions in the hemagglutination test with human sera might be explained by the presence of antibodies other than anti-M, presumably reacting with contaminating antigenic materials contained in the crude M preparations. Attempts to circumvent this difficulty by employing a more highly purified M preparation, supplied by Dr. Lancefield, were unsuccessful. Therefore, the possible blocking effect of heterologous M preparations on the cross-reactions was investigated. An illustrative experiment in which this was accomplished, and cross-reactions eliminated, is shown in Table V. The serum was from a patient convalescent from a type 24 streptococcal infection and contained bactericidal antibodies to type 24 streptococcus but not to type 14 streptococcus; there were, however, hemagglutinating antibodies reacting with both types. When 0.2 cc. of a 1:100 dilution of type 3 M protein was added to each tube, and the tubes incubated at 37° C. for one hour before the addition of the M protein-treated erythrocytes, the titer of type 24 antibodies was not altered but the reaction with cells treated with type 14 M was markedly inhibited.

Some preparations of M protein were more effective than others in the inhibition of nonspecific

TABLE IV

Agglutination of erythrocytes treated with tannic acid and types 24 and 14 M protein by sera from patients with type 24 streptococcal infections

Patient	Serum*	Agglutination titer	
		Type 24† M protein	Type 14 M protein
1	Acute	—‡	1:1,280
	conv.	1:640	1:640
2	Acute	—	1:640
	conv.	1:640	1:1,280
3	Acute	1:160	1:160
	conv.	1:640	1:2,560
4	Acute	—	—
	conv.	—	—
5	Acute	—	—
	conv.	1:280	—
6	Acute	1:40	1:80
	conv.	1:160	1:160

* Acute phase serum was obtained at the time of the streptococcal illness, convalescent serum approximately three weeks later.

† Erythrocytes were treated with 0.001 per cent tannic acid and a 1:250 dilution of M protein before being exposed to the serum dilutions.

‡ The figures are the highest dilution of serum that agglutinated the red cells. A negative reaction indicates that there was no hemagglutination at a 1:10 dilution or higher.

TABLE V

Effect on nonspecific hemagglutination of the addition of heterologous M protein and normal rabbit serum

Material added*	Serum dilutions													
	Type 24 antiserum and type 24 cells							Type 24 antiserum and type 14 cells						
	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280
Type 3 M protein†	3§	2	2	1	1	1	—	±	±	—	—	—	—	—
Saline	3	3	3	3	1	1	—	3	3	2	2	2	2	1
N.R.S.‡ and type 3 M protein	3	3	3	3	3	3	—	—	—	—	—	—	—	—
N.R.S.‡	3	3	3	3	3	2	—	3	3	3	3	3	3	—

* Material indicated added to each dilution of antiserum one hour before the addition of the treated erythrocytes.
† 0.2 cc. of a 1:100 dilution added to each serum dilution.
‡ Indicates normal rabbit serum; a 1 per cent solution in 0.75 per cent saline was used as the diluting fluid.
§ The numbers indicate the degree of agglutination of the red cells. 1 means slight but definite agglutination, and 2 and 3 mean more marked agglutination.

hemagglutination; one batch of type 3 M protein has been the most effective in experiments performed to this time. It should be noted that this batch of M protein apparently contains a large amount of non-M antigen since it has been associated with many nonspecific reactions when adsorbed to erythrocytes and tested with heterologous sera.

Boyden (19) reported that the stability of tannic-acid treated cells was improved by the use of normal rabbit serum in the diluting fluid. The effect of using 1 per cent rabbit serum in 0.75 per cent saline as the diluent for antiserum was therefore investigated in experiments dealing with the blocking effect of heterologous M protein. It was found that the degree of agglutination was much enhanced in mixtures of antisera with homologous M antigen, and the elimination of cross-reactions by blocking with heterologous M preparations was made more clear cut. The improvement of the test by normal rabbit serum is illustrated in Table V.

These methods for improving the specificity of the test are now being applied in tests of sera from patients with streptococcal infections and rheumatic fever, to be reported in a subsequent communication.

DISCUSSION

Attempts to bring about the adsorption of M protein to the surface of human erythrocytes, for the purpose of demonstrating anti-M protein antibody by hemagglutination, were unsuccessful when untreated red cells were employed. Kirby (16), in a study of the hemagglutination reaction with a mixture of streptococcal antigenic materials, was also unable to produce type-specific reactions indicating the presence of M protein on the erythrocytes.

The treatment of human group O erythrocytes with small amounts of tannic acid has made possible the adsorption to the cell surface of the M protein of group A streptococci. Erythrocytes so treated are selectively agglutinated by antistreptococcus rabbit sera and by the sera of humans convalescent from streptococcal infections. In the tests with human sera, however, non-specific cross-reactions with other types of M have occurred in a sufficiently high percentage of cases to make the use of the test, without modification, impractical.

The non-specific hemagglutination appeared to be due to an antibody reacting with unidentified antigenic material contained in the M protein preparations. The titer of this antibody did not appear to be related to the titer of specific M antibody. In some cases increased levels of both kinds of antibody were demonstrated in convalescent sera; in others, however, there was a rise in specific M antibody while both the acute-phase and convalescent sera showed equally high titers of heterologous hemagglutinating antibody.

The non-specific cross-reactions could be in-

hibited by the addition of a heterologous M preparation to the serum before mixing the latter with M-treated erythrocytes. Some batches of M were more effective than others; the more effective batches were those that seemed to contain relatively large amounts of non-specific antigenic material. The amount of heterologous antigen required to block cross-reactions was sometimes difficult to determine without detailed titrations, because of the occurrence of spontaneous agglutination in the presence of excess M protein. The addition of normal rabbit serum to the diluent considerably improved the test; specific agglutination was enhanced, and the inhibition of non-specific agglutination by heterologous M-preparations was more clear-cut. Studies are now in progress to determine if the test can be made practical for routine use by these modifications.

A possible solution to some of the problems in the hemagglutination test was the conversion of the test, by the addition of guinea pig complement, to one read by hemolysis. Attempts to do this were disappointing, since significant degrees of hemolysis occurred only in tests in which type 24 protein and antibody were employed. The reason for the failure to show hemolysis with tests involving other types of antigen and antibody is not known.

Much care was necessary in the treatment of erythrocytes with tannic acid and M protein. The spontaneous agglutination due to tannic acid could be prevented by determining the agglutinating property of each lot of tannic acid before use. It was more difficult to select the concentration of M protein that did not cause agglutination of the erythrocytes but was adsorbed in sufficient quantities to react with antibody. In using specific rabbit anti-sera this was not a great problem; the M antibodies were of such high levels that erythrocytes treated with very small amounts of antigen were agglutinated. With human sera, however, it was desirable to use as great a concentration of antigen as possible without causing spontaneous agglutination, in order to detect small amounts of antibody. Each batch of M protein required, therefore, detailed titrations to determine the optimal amounts for the preparation of sensitized cells.

SUMMARY

1. M Protein of group A streptococci was adsorbed to human group O erythrocytes previously exposed to small amounts of tannic acid, and these cells were specifically agglutinated by homologous rabbit antistreptococcus sera and sera of some patients convalescent from streptococcal infection.

2. The addition of guinea pig complement to the hemagglutination test resulted in significant specific hemolysis when type 24 M protein and antiserum were used; little or no hemolysis occurred when other types of M protein and antisera were involved.

3. Hemagglutination was specifically inhibited by the addition of homologous M protein to the serum before the addition of the M protein-sensitized erythrocytes.

4. In a study of human sera the appearance of type-specific hemagglutinins was demonstrated during convalescence from streptococcal infection. However, non-specific hemagglutination was frequently encountered. This could be inhibited in some instances by incubating the serum with a heterologous M preparation before the addition of the treated erythrocytes. Specific agglutination, and the inhibition of non-specific agglutination by heterologous M protein, were made more clear-cut by the use of normal rabbit serum in the diluting fluid.

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PULMONARY FUNCTION STUDIES IN MITRAL STENOSIS BEFORE AND AFTER COMMISSUROTOMY

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Decreased arterial oxygen saturation may occur in the blood of patients with mitral stenosis. This unsaturation might be due to impairment of diffusion of oxygen across the alveolar membrane or to impairment of ventilation-perfusion relationships in the lung. Both such abnormalities have been found in patients with mitral stenosis (1, 2, 3), but it is highly unlikely that appreciable unsaturation could occur during the breathing of room air as a result of impaired diffusion. This report deals with the pulmonary diffusing capacity and ventilation-perfusion relationships in nine patients with mitral stenosis before and after mitral commissurotomy.

MATERIAL AND METHODS

The patients were from the wards of Walter Reed Army Hospital and demonstrated evidence of uncomplicated mitral stenosis which had led to progressive dyspnea and/or hemoptysis. In each instance the diagnosis was confirmed at the time of commissurotomy.

The patients were studied in the fasting state during the inspiration of room air and a low oxygen mixture. Expired air was collected for a three-minute interval in the midst of which arterial blood was allowed to flow from an indwelling radial arterial needle for a one-minute period into a syringe containing mercury and a minimum of heparin. Arterial gas tensions were measured immediately by the direct method of Riley, Proemmel, and Franke (4) which has been standardized in this laboratory (5). Expired gas volumes were measured in a Tissot spirometer and the composition was measured by means of the Scholander micro-gas analyzer (6). Alveolar oxygen tension was calculated from the alveolar equation, substituting the arterial pCO_2 for the alveolar pCO_2. The oxygen diffusing capacity (D_{O_2}) and the percentage venous admixture ($\dot{Q}va/\dot{Q}t$) as defined in the expanded sense by Riley and Cournand (7, 8) were calculated, assuming a 25 per cent difference in saturation between end-capillary and mixed venous blood ($P^*_c - P_{\bar{v}}$). In Cases 4 and 5 a 30 per cent difference in saturation was assumed because of the large amount of venous admixture.

RESULTS

The results of these studies are presented in Table I. In normal subjects, not shown here, the venous admixture averages 3 per cent, varying from 0–8 per cent. Also, in normal subjects, the alveolar-arterial oxygen gradient during the inspiration of a low oxygen mixture is negligible so that the lower limit of normal for D_{O_2} has arbitrarily been selected as 20 (9). Since the methods employed do not measure gradients with an accuracy of more than 3–4 mm. Hg, and since accurate calculation of the diffusing capacity is impossible in the presence of small gradients, D_{O_2} is simply listed as "normal" if the effective alveolar-end-capillary gradient ($P^*_A - P^*c_{O_2}$) during the inspiration of the low oxygen mixture is less than 4 mm. Hg. In all but three patients the per cent venous admixture was increased, ranging from 8–18 per cent. In all but one instance this became normal after pulmonary congestion was relieved by commissurotomy. Since the arteriovenous difference may become less after operation, and since the estimated amount of venous admixture would err by 3 per cent if the arteriovenous difference varied by 10 per cent from the assumed value, only a change in venous admixture of over 3 per cent may be considered significant. Thus, four out of the six patients with increased venous admixture preoperatively showed a significant fall after operation.

Five of the patients showed resting hyperventilation which, in four, disappeared after commissurotomy. In the patient (Case 7) in whom the hyperventilation and elevated venous admixture effect persisted there was indication of psychological abnormality such that the hyperventilation appeared to have been functional and may account for part of the increase in venous admixture (5).

Oxygen diffusing capacity was not measured in three of the patients preoperatively. In the others, it was reduced in two instances. In Case 2 there was a postoperative increase in diffusing capacity whereas diffusing capacity fell postoperatively in Case 1. The other patients showed slight or no change in this function.

TABLE I

Physiological data on patients with mitral stenosis pre- and post-operatively

Case	Age	B.S.A. *sq.m.*	Sex	Pre or postop.	$P_{I_{O_2}}$ *mm. Hg*	$P_{a_{CO_2}}$ *mm. Hg*	$P_{a_{O_2}}$ *mm. Hg*	R	$\dot{V}$ *L./min.* BTPS	$P^e_{A_{O_2}}$ *mm. Hg*	$P^e_{C_{O_2}}$ *mm. Hg*	$\dot{Q}va/\dot{Q}t.$ ×100	D_{O_2} *cc./min./ mm.*	D.S./TV ×100	$\dot{V}_{O_2}$ *L./min.* STPD
1	41		M	Preop.	146	30	89	.84		112	112	9			
					146	30	84	.88		113	113	13			
					95	30	53	.86		62	59	(13)	Normal		
				3 weeks	148	34	95	.91	7.02	111	111	6		10	210
				postop.	96	34	49	.94	8.03	60	51	(6)	11	19	202
2	18	1.38	F	Preop.	150	34	88	.73	9.04	106	105	8		34	253
					97	30	48	.90	10.95	64	50	(8)	9	39	221
				12 weeks	100	40	46	.70	7.86	45	45	(2)	Normal	33	259
				postop.	149	42	91	.72	5.52	94	94	2		30	206
3	19	1.75	M	Preop.	148	37	94	.81	6.48	104	104	5		19	241
					96	34	52	.92	7.63	59	54	(5)	16	14	247
				7 weeks	149	37	92	.84	5.67	106	106	6		16	206
				postop.	100	37	50	.83	5.72	56	52	(6)	15	18	198
					71	28	37	1.01	9.26	43	37	(6)	20	15	222
4	38	1.58	F	Preop.	148	32	73	.78	9.27	109	109	17		38	230
					148	32	57	.70	9.72	105	105	33		40	251
				2½ weeks	149	37	83	.80	5.41	105	105	11		28	173
				postop.	97	31	53	.91		66	58	(11)	8		163
5	27	1.52	F	Preop.	148	37	68	.90	8.26	108	100	18*		32	214
					148	38	70	.86	7.96	105	95	15*		32	227
				3 weeks	149	42	84	.89	6.76	103	92	5		33	190
				postop.	149	43	78	.85	6.75	100	88	7		36	191
					101	36	39	1.20	9.47	70	39	(7)	4	38	165
6	35	1.76	F	Preop.	149	39	90	.75	5.54	100	100	5		23	232
					101	37	62	.92	5.98	61	64	(5)	Normal	23	204
				6½ weeks	148	34	90	.70	5.54	102	102	6		18	213
				postop.	70	29	39	1.00	6.22	41	40	(6)	Normal	22	117
7	25	1.38	F	Preop.	148	30	92	.80	7.25	112	112	8		25	192
					148	32	90	.81	6.52	110	110	9		25	181
				5 weeks	97	31	52	.81	7.47	60	56	(10)	14	29	191
				postop.	149	30	87	.73	8.26	110	110	10		25	199
8	41	1.47	F	Preop.	101	36	55	.98	7.70	61	58	(4)	Normal	30	162
					149	37	96	.80	6.76	105	105	4		28	182
				3½ weeks	149	38	92	.83	4.64	105	105	6		21	113
				postop.	100	36	57	.90	7.11	60	60	(6)	Normal	26	180
					71	31	35	.97	8.51	39	35	(6)	23	26	185
9	24	1.86	M	Preop.	148	33	89	.84	8.66	110	110	9		21	261
					100	33	51	.78	8.01	59	54	(9)	20	24	297
				3 weeks	149	36	90	.73	6.67	103	103	6		20	250
				postop.	90	37	42	.78		43	43	(6)	Normal		263

* $P^e_A - P\bar{c}_{O_2}$ of 37 assumed for calculation of per cent venous admixture, since that was the mean alveolar-capillary gradient determined postoperatively.

DISCUSSION

Certain effects of longstanding mitral stenosis are apparent from these studies. As reported also by others (2, 3) the pulmonary diffusing capacity for oxygen is frequently reduced, although usually not greatly enough to cause an alveolar-arterial oxygen gradient during the inspiration of room air. Four of our patients showed a very low oxygen diffusing capacity postoperatively, but in only one instance (Case 5) did the impaired diffusion cause an appreciable alveolar-arterial gradient during the breathing of room air. This impairment is not likely to be greatly improved by commissurotomy and may be attributed either to thickening of the alveolar capillaries, as has been shown to occur in mitral stenosis (10) or to reduction in the size of the effective pulmonary capillary bed.

The impaired distribution of blood and gas in

the lung, which is largely eliminated by reduction of the pulmonary hypertension, may be attributed to pulmonary congestion with effective blocking of the small airways by transudate. A similar effect has been observed in experimental pulmonary edema (11), in which there is a large venous admixture effect but no impairment of diffusion across the alveolar membrane. Prolonged rest in the supine position is apt to increase the pulmonary congestion. This is exemplified by Case 4 in whom the venous admixture increased from 17–33 per cent as the patient developed mild dyspnea after lying supine for one hour. There was a good correlation between the size of the venous admixture effect and the clinical symptomatology.

The frequent occurrence of resting hyperventilation which disappears after commissurotomy suggests that the engorged pulmonary bed serves as a reflex stimulus to ventilation.

Ventilatory studies were performed on these patients and revealed normal or slightly reduced maximum breathing capacity and vital capacity in each case. Only in the patient with a 33 per cent venous admixture effect was the lung dead space greatly increased (40 per cent of the tidal volume), and this fell postoperatively. A large dead space effect would be expected when large numbers of alveoli are not effectively ventilated. The small increase in dead space in Cases 2 and 5 was not significantly changed postoperatively.

SUMMARY

Pulmonary function has been studied in nine cases of mitral stenosis before and after commissurotomy. Several cases showed an increase in the effective shunting of venous blood through unaerated or poorly ventilated lung with, in some instances, arterial unsaturation. This abnormality was largely corrected by commissurotomy. Pulmonary diffusing capacity was frequently impaired, little affected by operation but usually not sufficiently reduced to cause lowering of arterial oxygen tension during the breathing of room air. Resting hyperventilation, when due to pulmonary congestion, was diminished after commissurotomy.

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OXYGEN TENSION OF TISSUES BY THE POLAROGRAPHIC METHOD. VI. EFFECT OF CHANGES IN POSITION ON OXYGEN TENSION OF THE SKIN OF TOES[1]

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INTRODUCTION

It is generally believed that the ischaemia of peripheral arterial occlusion is aggravated by raising the limb and relieved somewhat by lowering the limb. Patients with this condition often complain of foot pain while in bed. This is relieved by getting out of bed. If the head of the bed is raised or special devices are used to lower the leg (1) the pain of the foot is often relieved and the color of the foot improved. Conversely, a case recently came to our attention in which the foot of a patient, who had been confined to bed for a year because of a cerebrovascular accident, became gangrenous only two days after being elevated on two pillows.

Elevation of a normal limb decreases the arterial pressure within it, whereas dependency increases the pressure. Measurements of intra-arterial pressure show that the pressure varies directly with the vertical distance from the right atrium to the point of measurement (2). Such changes might be expected to cause predictable changes in limb blood flow, but the situation is complicated by concomitant changes in venous pressure (3, 4, 5, 6, 7). In limbs with obstructive vascular disease the situation is still more complicated because of the marked reduction in arterial pressure which occurs distal to an obstruction (8).

Measurements of blood flow have been made in limbs at different levels of elevation and dependency; most investigators found the blood flow to increase when the limb is lowered and decrease when the limb is elevated (9, 10, 11, 12). One group found flow to decrease on dependence (13), and one to vary depending upon the preceding position (14). Some of the observations were complicated because the change in position of the limbs was accompanied by changes in the disposition of the body, and by differences in muscular tension in the limbs (12). The various conclusions are probably dependent in part upon the degree and manner of changes of position, and upon the methods used to measure blood flow.

We have chosen to measure the oxygen tension of the skin of feet made severely ischaemic by peripheral arterial occlusion and to study changes in oxygen tension resulting from slight changes in the angle of the bed from the horizontal. Comparable studies were made on normals. Since there is no reason to suppose that a small change in position alters cutaneous metabolism, a change in oxygen tension of the skin may be taken as an index of a changed supply of oxygen, and presumably of other substances from the blood.

Some studies of prolonged maintenance of the dependent position were made in order to make sure that the tension changes are sustained.

Studies were made of normal and ischaemic limbs with unchanged position but with venous pressure increased by a cuff. The cuff pressure was equal to the hydrostatic pressure that had resulted, in the previous experiments, by lowering the limb.

METHOD

During the experiments the average room temperature was 26.7° C., the extremes 20.4° C. and 29.1° C. The average variation of room temperature during an experiment was 1.6° C., the greatest variation was 2.7° C. A tilt table 6.5 feet in length was used. It was covered by a 1.5 inch mattress with a pillow. Enough blankets were provided to maintain a state of mild peripheral vasodilatation. The foot end of this table was raised or lowered without causing apprehension.

Four open tip platinum electrodes (15, 16) were used in each experiment. They were inserted into the skin at the base of the first, second, fourth, and fifth toes, in the areas where the thermocouples were placed. The method of estimating oxygen tension was that previously described

[1] This investigation was supported by a research grant (392 C3) from the National Heart Institute of the National Institutes of Health, Public Health Service.

TABLE I

*Difference between oxygen tension of normal and of ischaemic tissues as measured by two electrodes**

Electrode number	Number of expt's	Number of det's	Relative position of foot	Mean galvanometric readings			Standard deviation of difference-means
				Normal	Ischaemic	Diff.	
2	10	40	level	10	3.9	6.1	1.0
3	10	40	level	8.8	4.2	4.6	0.7
2	10	24	lowered	9.7	6.7	3.0	1.8
3	10	24	lowered	8.4	5.0	3.4	1.1
2	10	10	raised	9.5	3.1	6.4	2.2
3	10	10	raised	8.6	3.5	5.1	1.4

* The oxygen tension was significantly higher (P = <5%) in the normal than in the ischaemic foot except in the case of electrode Number 2 when used in the lowered foot.

in this Journal (16). The apparatus consists of a platinum cathode, a relatively simple circuit, variable resistances and meters to control and measure voltage and current, and a calomel half cell as an anode.

At voltage 0.4 to 0.8 current varies linearly with oxygen tension at the electrode tip. In the present study each result is reported as a percentage difference of current from that obtained in the horizontal position.

Room temperature and skin temperatures at electrode sites were recorded just before each change in position. Galvanometric readings from each electrode were taken every two minutes. In order to make certain that each electrode was responsive, oxygen was briefly administered by inhalation and the response in galvanometric readings noted.

In the case of the normal subjects and of six of the patients, the readings were then continued with the subject for twenty minutes in each of the following positions: horizontal, foot of table lowered 7 inches, horizontal, lowered 7 inches, horizontal, foot of table raised 7 inches, and horizontal. This 7 inch deviation from the horizontal entails a hydrostatic pressure difference of 4 to 5 inches between the right atrium and toe. In six other patients, after the initial twenty minute control period, the foot-down position was maintained for two hours. They were then returned to the horizontal for a final twenty minutes. In these prolonged experiments no foot-up position was used.

Fourteen patients with severe peripheral arteriosclerosis with occlusion were studied. Their ages ranged from 52 to 80 years. Twelve were males, two females. Five were diabetic, and in two cases a lumbar ganglionectomy had been performed. On completion of a heat reflex vasodilatation test in a 20° C. constant temperature room the skin temperature at the base of the toes averaged 25.7°C. (range 20.2 to 28.6° C.). The average temperature under these conditions in the normal toe is 31° C. Two of the electrodes were used throughout all experiments. The average of the galvanometric readings with these electrodes in the ischaemic feet in any one position was much less than the average in normal feet in the same position (Table I). Further evidence of the severity of the arterial occlusion is given by the slowness with which inhaled oxygen was detectable in the toes. In the normals the oxygen tension increased by 144 per cent (average) within two minutes, whereas in the ischaemic limbs it increased by 11 per cent (average). This delay in the abnormal limbs is seen in normals only when there is severe vasoconstriction (17). The eight normal subjects, six female and two male, ranged in age from 16 to 48 years.

In an attempt to learn whether the increased venous pressure incident to the foot-down position might account for the oxygen tension changes observed, experiments were conducted in which venous pressure was raised by a pressure cuff on the ankle. Two patients and two normal subjects were studied in the horizontal position. After a twenty minute study, a cuff pressure of 13 mm. Hg was maintained for twenty minutes. This sequence was then repeated. Skin oxygen tension and temperature were recorded as before.

RESULTS

In patients and normals, significant increases of oxygen tension were observed in the foot-down position (Table II). The increases were greater, both relatively and absolutely, in patients (average 13 per cent) than in normals (average 4 per cent). When the foot-down position of patients was main-

TABLE II

*Changes in cutaneous oxygen tension in feet resulting from changes in position**

Position of foot	Number of expt's	Number of det's	% Mean diff. in O_2 tension from horizontal	Standard deviation
Lowered 20 mins.				
Normal	6	47	+ 4	.16
Ischaemic	6	39	+13	.17
Lowered 120 mins.				
Ischaemic	6	44	+22	.16
Raised 20 mins.				
Normal	6	47	insignificant	.15
Ischaemic	6	34	−22	.25

* A significant change (P = <5%) in oxygen tension is noted in all cases except in raising a normal extremity for 20 minutes.

tained for two hours, rather than for twenty minutes, there were further increases in oxygen tension, and on return to the horizontal position the oxygen tension decreased promptly and completely. The foot-up position resulted in significant decreases in oxygen tension in the case of the patients (average 22 per cent), but in the case of normals no significant change in oxygen tension resulted. In the patients pain usually occurred when the legs were elevated but never in the case of normals.

The temperature measurements of skin showed no statistically significant change in blood flow in any series of experiments.

When a pressure cuff (13 mm. Hg, or 7 inches water) was used on the ankles of four subjects, no statistically significant changes in oxygen tension or skin temperature resulted.

DISCUSSION

The increase in oxygen tension of the skin of the feet when dependent and its decrease on elevation may result from slight changes in blood flow, although blood flow changes were not detectable by the skin temperature measurements. It is possible that part of the oxygen tension changes were caused by capillaries being more distended in the dependent position and collapsed in the elevated position. The distension might facilitate the passage of oxygen to the tissue. However, we could demonstrate no change in oxygen tension, in the horizontal position, resulting from a cuff pressure of 7 inches of water (13 mm. Hg). It is possible that this small cuff pressure reduced blood flow (18) (but we were unable to detect it), that transfer of oxygen from the blood to the tissues was facilitated, and that the two opposing effects explain the lack of change of oxygen tension in the cuff experiments.

All the methods of measuring blood flow have some disadvantage when used in measuring posturally-induced changes. Oxygen content of venous blood returning from a limb may fail to show the state of the most severely ischaemic (12, 14) part, since the sampling must be from the larger vessels far proximal to that part. Skin temperature measurement (9, 10, 13) at reasonably constant room temperature is an indication of blood flow at the spot being observed, but by this method we were unable to demonstrate changes in flow in the present experiments. Pulse wave plethysmography is completely unsuitable as an index of change in blood flow induced by change in posture (8). Cuff-plethysmography is difficult or impossible when the capacity vessels are full (19), as in the dependent position. In the elevated position, especially in limbs made ischaemic by arterial occlusion, great care must be taken to avoid interference with flow. We know of no measurements of posturally-induced circulation changes by calorimeter and believe that they would be open to the same criticism as that made of skin temperature measurements.

We think that the change in nutrition of ischaemic tissues brought about by change in posture is shown better by these measurements of oxygen tension than by measurements of blood flow.

SUMMARY

Twelve patients with ischaemic feet and six normal individuals were tilted for twenty minutes from the horizontal position to the 7 inch foot-down position, and for the same period of time to the 7 inch foot-up position. The cutaneous oxygen tension at the base of the ischaemic toes was found to be much less than that in the toes of normal subjects.

On lowering the ischaemic feet, there was an average increase of oxygen tension of 13 per cent, and on elevating them from the horizontal, a decrease of 22 per cent. In normal individuals there was a slight increase of oxygen tension upon lowering the feet, and no significant change on raising the feet. The increases in oxygen tension were maintained or further increased in the toes of patients who remained for two hours in the foot-down position.

The changes in oxygen tension incident to change in position may have resulted from undetected changes in blood flow, from changes in effective capillary surface, or from other factors. There is no reason to suppose that they resulted from changes in cutaneous oxygen utilization. A foot-down position of this degree increases the oxygen, and presumably other hematogenous substances, available to the skin. This may explain the relief of pain and the improvement in skin color that often result from placing an ischaemic foot in a somewhat dependent position, and gives an objective reason for using the dependent position of the foot therapeutically.

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THE EFFECTS OF VENOUS CONGESTION OF THE LIMBS UPON RENAL CLEARANCES AND THE EXCRETION OF WATER AND SALT. I. STUDIES IN NORMAL SUBJECTS AND IN HYPERTENSIVE PATIENTS BEFORE AND AFTER SPLANCHNICECTOMY [1, 2]

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INTRODUCTION

The assumption of the upright position is known to cause decreases in urine volume, renal blood flow, and sodium excretion (2, 3). Since, by congesting the limbs of supine subjects, it is possible to simulate under better controlled conditions certain of the hemodynamic effects of the upright position while avoiding others, it seemed worthwhile to investigate the effects of this procedure upon renal clearances and the urinary excretion of salt and water. It seemed particularly pertinent to do so in hypertensive patients before and after lumbodorsal splanchnicectomy, an operation which conceivably might alter the renal responses to venous congestion. It also seemed relevant to inquire whether the renal disturbances caused by this procedure in any way resemble those found in association with other conditions characterized by venous congestion or edema in the limbs.

SUBJECTS AND METHODS

The subjects were patients admitted to the Massachusetts Memorial Hospitals, who at the time of study had either no cardiovascular-renal abnormalities (normotensive controls) or essential hypertension (hypertensives before and after splanchnicectomy). Throughout the test the subjects were given by mouth 200 cc. of water every half hour. Otherwise they were studied in the basal state, lying supine in bed. At the beginning of the test, blood pressure cuffs were applied smoothly to the upper thighs and bandaged (without compression) in place. A soft rubber catheter was placed in the bladder in a position that allowed complete drainage. A freshly prepared infusion containing 4 per cent glucose solution in water and appropriate amounts of inulin and sodium para-aminohippurate for renal clearance studies (4) was given at a rate of 4 cc. per minute in an antecubital vein. An indwelling needle, fitted with obturator for taking blood samples, was inserted through the skin anesthetized with metycaine into the brachial artery, or in a few experiments into a peripheral vein. Arterial pressure and pulse rate were measured by the usual clinical methods in the opposite arm. Accurately measured collections of urine were made and the bladder was washed with 20 cc. distilled water at (usually) 10-minute intervals and when desired, samples

[1] Presented in part at the Forty-First Annual Meeting of the American Society for Clinical Investigation, May 2, 1949 (1).

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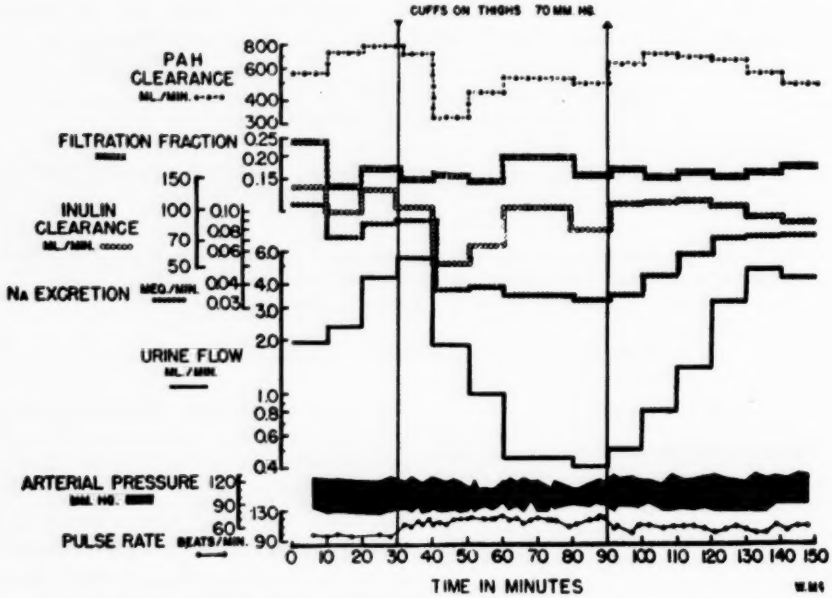

FIG. 1. CHART OF PAH CLEARANCE, FILTRATION FRACTION, INULIN CLEARANCE, SODIUM EXCRETION, URINE FLOW, ARTERIAL PRESSURE AND PULSE RATE IN AN EXPERIMENT ON A NORMOTENSIVE SUBJECT WHOSE LIMBS WERE CONGESTED BY INFLATING CUFFS ON THE THIGHS FOR 60 MINUTES

The ordinate scales are logarithmic except those for arterial pressure and pulse rate.

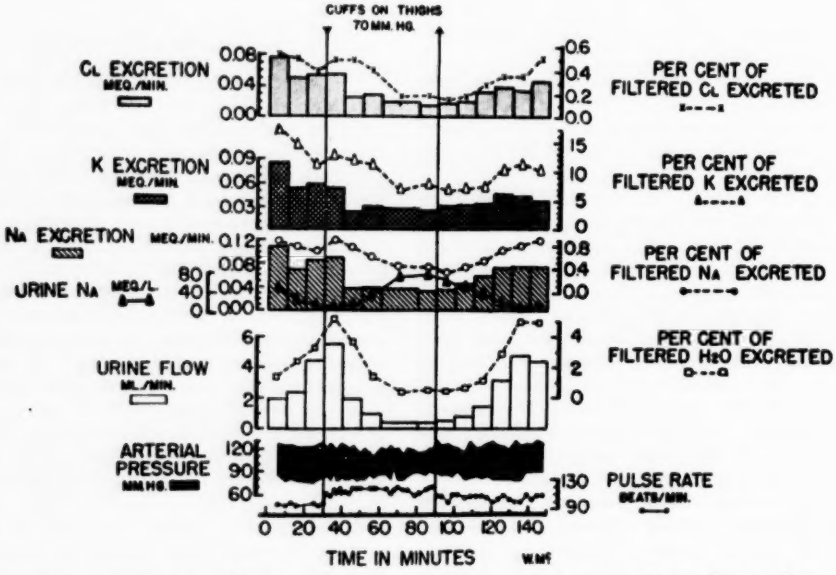
CUFFS ON THIGHS 70 MM. HG.
Cl EXCRETION MEQ./MIN.
K EXCRETION MEQ./MIN.
Na EXCRETION MEQ./MIN.
URINE Na MEQ./L
URINE FLOW
ARTERIAL PRESSURE MM. HG.
PER CENT OF FILTERED Cl EXCRETED
PER CENT OF FILTERED K EXCRETED
PER CENT OF FILTERED Na EXCRETED
PER CENT OF FILTERED H2O EXCRETED
PULSE RATE BEATS/MIN.
TIME IN MINUTES

FIG. 2. CHART OF THE CHLORIDE, POTASSIUM AND SODIUM EXCRETIONS, URINE SODIUM CONCENTRATION, URINE FLOW, AND CALCULATED PER CENT OF FILTERED SUBSTANCES EXCRETED (E/F) IN THE EXPERIMENT SHOWN IN FIGURE 1

The ordinate scales are all arithmetic.

of blood were taken 2½ minutes later. After a good (3 to 10 cc. per min.) and fairly steady urine flow was established, and when at least three control renal clearance periods had elapsed, the cuffs on the thighs were inflated at slightly less than diastolic pressure (usually at 70 mm. Hg). Observations were made for one to six periods, whereupon the congesting cuffs were released and recovery observations were made.

In some experiments during the control period before, and the recovery period after, venous congestion of the limbs, the thigh cuffs were inflated every 5 minutes at the same pressure as used during the congestion period, but only for 30 seconds. This was to accustom the patient to the sensory stimulus of the inflated cuff. In other experiments, after a suitable control period the entire limbs up to the thigh cuffs were *compressed* by means of a series of cuffs or inflatable leggings (of a "G"-suit) for the same length of time as they were subsequently (after another control period) congested by inflation of the uppermost cuff alone. This compression was designed to cause an equal or greater degree of ischemia, anoxia, and discomfort in the limbs without venous congestion or edema. A number of such additional experiments were done in order to test the effects of other variations in the standard procedures just described upon urine flow, electrolyte excretion, arterial pressure, and pulse rate. These will not be described in detail, but will be mentioned under "Special Results."

Samples of urine and blood were analyzed for clearance substances according to the methods of Goldring and Chasis (4). They were analyzed for sodium and potassium by means of an internal standard flame photometer [9] and for chloride by the method of Wilson and Ball (5).

Hematocrits of the blood samples were measured in Wintrobe tubes, and the protein content of the plasma by a specific gravity technique (Copper Sulfate).

RESULTS

General

Detailed results on a number of patients are shown in the Figures and in Table I, and a statistical analysis of these results is given in Table II. In the experiments shown in Table I the various periods have been grouped and averaged in an attempt to save space without altering significantly

[9] Perkin-Elmer Corporation, Main Avenue, Norwalk, Connecticut.

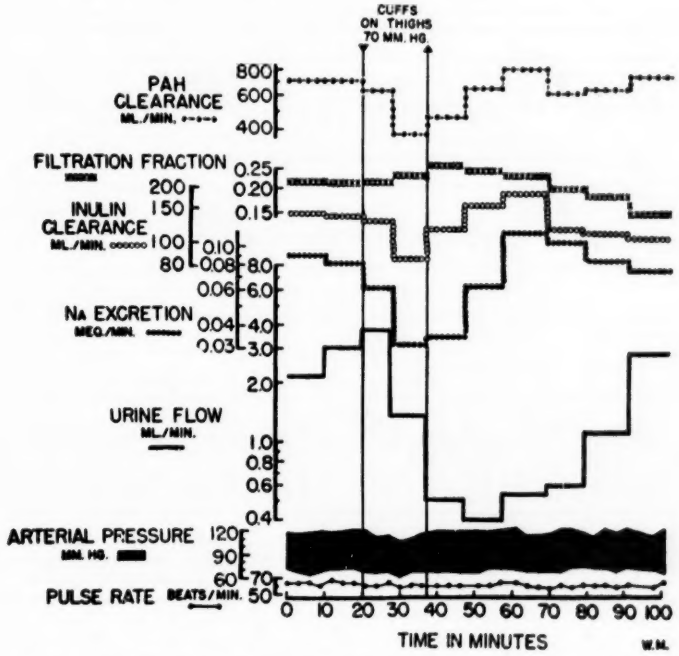

FIG. 3. CHART OF AN EXPERIMENT ON A NORMOTENSIVE SUBJECT CONDUCTED SIMILARLY TO THAT SHOWN IN FIGURE 1 EXCEPT THAT THE LIMBS WERE CONGESTED FOR A SHORTER (18 MINUTES) PERIOD

Method of charting as in Figure 1.

TABLE I-A—*Normotensive subjects*

Patient Age Sex and Surface area (sq. m.)	Observations		Periods before congestion				Periods during congestion						Periods after congestion					
			Fourth and more		First through third		First		Second and third		Fourth and more		First		Second and third		Fourth and more	
	Function	Unit	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N
33 G. V. F 1.54	B.P.	mm. Hg			106/74	10	108/72	10	114/79	15	119/84	10	117/83	4	124/80	6	127/81	6
	P.R.	beats/min.			59	10	61	10	67	15	69	10	63	4	59	6	60	6
	UV	ml./min.			3.4	3	4.3	1	0.59	2	0.35	2	1.0	1	2.8	2	5.9	2
	C_{IN}	ml./min.			83	3	85	1	71	2	88	2	120	1	104	2	90	2
	C_{PAH}	ml./min.			410	3	409	1	316	2	344	2	457	1	461	2	418	2
	UNaV	mEq./min.			0.080	3	0.087	1	0.068	2	0.075	2	0.083	1	0.095	2	0.085	2
	UClV	mEq./min.			0.055	3	0.055	1	0.043	2	0.045	2	0.046	1	0.053	2	0.051	2
	UKV	mEq./min.			0.023	3	0.018	1	0.013	2	0.017	2	0.023	1	0.026	2	0.022	2
26 M. C. F 1.48	B.P.	mm. Hg	117/71	10	117/72	11	111/72	3	106/74	7	110/79	10	113/74	4	112/73	7	112/71	6
	P.R.	beats/min.	97	10	93	11	104	3	108	7	111	10	102	4	96	7	90	6
	UV	ml./min.	1.9	3	8.3	3	6.4	1	0.70	2	0.34	2	0.30	1	0.35	1	0.50	1
	C_{IN}	ml./min.	102	3	93	3	78	1	37	2	57	2	67	1	74	1	82	1
	C_{PAH}	ml./min.	664	3	659	3	506	1	270	2	364	2	392	1	510	1	622	1
	UNaV	mEq./min.	0.22	3	0.24	3	0.20	1	0.072	2	0.042	2	0.027	1	0.036	1	0.060	1
	UClV	mEq./min.	0.15	3	0.15	3	0.12	1	0.046	2	0.029	2	0.024	1	0.027	1	0.048	1
	UKV	mEq./min.	0.036	3	0.046	3	0.036	1	0.015	2	0.020	2	0.023	1	0.026	1	0.034	1
38 W. Mc. M 1.81	B.P.	mm. Hg			125/80	7	122/82	8	117/83	14	118/86	15	128/85	6	122/84	6	125/84	10
	P.R.	beats/min.			97	7	112	8	118	12	116	14	106	5	108	6	105	10
	UV	ml./min.			2.9	3	5.6	1	1.5	2	0.43	2	0.50	1	1.1	2	4.1	3
	C_{IN}	ml./min.			123	3	107	1	58	2	92	2	110	1	114	2	98	3
	C_{PAH}	ml./min.			694	3	714	1	385	2	517	2	656	1	725	2	586	3
	UNaV	mEq./min.			0.089	3	0.090	1	0.038	2	0.034	2	0.035	1	0.052	2	0.073	3
	UClV	mEq./min.			0.060	3	0.054	1	0.027	2	0.018	2	0.018	1	0.027	2	0.041	3
	UKV	mEq./min.			0.066	3	0.054	1	0.027	2	0.027	2	0.031	1	0.033	2	0.043	3
39 R. P. F 1.66	B.P.	mm. Hg			111/73	10	119/75	8	119/79	16	117/79	9	113/78	11	110/76	9	111/74	14
	P.R.	beats/min.			72	10	79	8	85	18	97	21	88	11	73	9	74	14
	UV	ml./min.			5.1	3	6.2	1	2.4	2	0.53	3	0.28	1	0.30	1	4.1	4
	C_{IN}	ml./min.			133	3	152	1	84	2	75	3	91	1	110	1	203	4
	C_{PAH}	ml./min.			689	3	748	1	420	2	369	3	422	1	490	1	957	4
	UNaV	mEq./min.			0.13	3	0.17	1	0.11	2	0.072	3	0.066	1	0.057	1	0.11	4
	UClV	mEq./min.			0.11	3	0.14	1	0.090	2	0.067	3	0.063	1	0.051	1	0.095	4
	UKV	mEq./min.			0.033	3	0.039	1	0.023	2	0.019	3	0.024	1	0.026	1	0.058	4
19 L. B. F 1.41	B.P.	mm. Hg			114/81	6	115/83	10	110/82	14	110/84	16	118/88	5	113/80	5	111/79	5
	P.R.	beats/min.			86	5	88	10	89	14	91	16	80	5	79	5	86	5
	UV	ml./min.			9.6	3	3.1	1	2.2	2	1.7	3	1.8	1	3.5	2	5.5	2
	C_{IN}	ml./min.			88	3	99	1	80	2	84	3	90	1	92	2	91	2
	C_{PAH}	ml./min.			398	3	417	1	339	2	302	3	348	1	379	2	331	2
	UNaV	mEq./min.			0.092	3	0.098	1	0.044	2	0.026	3	0.030	1	0.067	2	0.076	2
	UClV	mEq./min.			0.049	3	0.084	1	0.034	2	0.017	3	0.020	1	0.047	2	0.065	2
	UKV	mEq./min.			0.028	3	0.024	1	0.020	2	0.019	3	0.023	1	0.026	2	0.030	2

TABLE I-A—*Continued*

Patient Age and Sex Surface area (sq. m.)	Observations		Periods before congestion				Periods during congestion						Periods after congestion					
			Fourth and more		First through third		First		Second and third		Fourth and more		First		Second and third		Fourth and more	
	Function	Unit	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N
35 R. F.* F 1.41	B.P.	mm. Hg			104/68	6	105/74	6	101/70	16			102/79	6	105/77	18		
	P.R.	beats/min.			75	6	70	6	64	6			74	1	61	7		
	UV	ml./min.			8.2	2	8.6	1	6.8	2			5.8	1	6.8	2		
	C_{IN}	ml./min.			101	2	87	1	90	2			87	1	97	2		
	C_{PAH}	ml./min.			716	2	637	1	576	2			574	1	588	2		
	UNaV	mEq./min.			0.13	2	0.20	1	0.14	2			0.12	1	0.19	2		
	UClV	mEq./min.			0.069	2	0.088	1	0.059	2			0.039	1	0.061	2		
	UKV	mEq./min.			0.032	2	0.048	1	0.036	2			0.037	1	0.063	2		
35 R. F.† F 1.41	B.P.	mm. Hg			104/78	24	101/76	12	99/77	18			102/79	7	102/77	6		
	P.R.	beats/min.			63	8	73	4	70	7			69	7	73	6		
	UV	ml./min.			6.5	3	6.7	1	2.2	2			1.0	1	4.3	2		
	C_{IN}	ml./min.			94	3	93	1	36	2			63	1	134	2		
	C_{PAH}	ml./min.			583	3	608	1	238	2			389	1	821	2		
	UNaV	mEq./min.			0.17	3	0.21	1	0.077	2			0.078	1	0.19	2		
	UClV	mEq./min.			0.054	3	0.061	1	0.022	2			0.024	1	0.046	2		
	UKV	mEq./min.			0.055	3	0.073	1	0.027	2			0.029	1	0.042	2		
45 C. H. M 1.90	B.P.	mm. Hg			114/74	11	114/82	10	111/81	10			115/80	4	114/80	6	112/78	10
	P.R.	beats/min.			71	10	77	10	80	10			74	4	71	6	70	9
	UV	ml./min.			3.3	3	4.2	1	3.3	1			1.7	1	2.6	2	3.4	3
	C_{IN}	ml./min.			108	3	90	1	81	1			70	1	99	2	105	3
	C_{PAH}	ml./min.			685	3	568	1	486	1			403	1	568	2	540	3
	UNaV	mEq./min.			0.23	3	0.21	1	0.16	1			0.11	1	0.18	2	0.18	3
	UClV	mEq./min.			0.19	2	0.17	1	0.13	1			0.10	1	0.16	2	0.14	2
	UKV	mEq./min.			0.052	3	0.045	1	0.038	1			0.028	1	0.039	2	0.039	3
31 M. B. F 1.472	B.P.	mm. Hg	113/76	9	118/80	8	118/88	7	120/89	6			120/85	4	120/87	5	123/86	7
	P.R.	beats/min.	104	9	101	8	105	6	110	6			108	4	104	5	106	7
	UV	ml./min.	1.6	3	4.2	3	10	1	2.2	1			0.70	1	0.85	2	1.8	3
	C_{IN}	ml./min.	102	3	123	3	113	1	88	1			104	1	149	2	119	3
	C_{PAH}	ml./min.	472	3	434	3	416	1	321	1			352	1	475	2	438	3
	UNaV	mEq./min.	0.090	3	0.12	3	0.18	1	0.13	1			0.10	1	0.14	2	0.12	3
	UClV	mEq./min.	0.059	3	0.080	3	0.094	1	0.084	1			0.066	1	0.084	2	0.089	3
	UKV	mEq./min.	0.016	3	0.024	3	0.027	1	0.023	1			0.017	1	0.021	2	0.024	3

* First congestion in one experiment on R. F.
† Second congestion in same experiment on R. F.

TABLE I-A—*Continued*

Patient Age and Sex Surface area (sq. m.)	Observations		Periods before congestion				Periods during congestion						Periods after congestion					
			Fourth and more		First through third		First		Second and third		Fourth and more		First		Second and third		Fourth and more	
	Function	Unit	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N
44 I. B. F 1.855	B.P.	mm. Hg	125/78	22	122/78	8	123/82	7	122/83	7			133/87	3	132/86	6	125/80	2
	P.R.	beats/min.	83	22	82	8	90	6	89	7			85	3	89	6	90	2
	UV	ml./min.	5.6	7	3.2	3	3.8	1	1.1	1			0.90	1	3.1	2	4.7	1
	C_{IN}	ml./min.	111	7	104	3	89	1	61	1			115	1	149	2	120	1
	C_{PAH}	ml./min.	545	7	543	3	434	1	292	1			474	1	573	2	470	1
	UNaV	mEq./min.	0.22	7	0.21	3	0.20	1	0.11	1			0.15	1	0.23	2	0.21	1
	UClV	mEq./min.	0.18	7	0.17	3	0.15	1	0.091	1			0.12	1	0.17	2	0.16	1
	UKV	mEq./min.	0.042	7	0.039	3	0.037	1	0.023	1			0.030	1	0.038	2	0.031	1
26 J. B. F 1.57	B.P.	mm. Hg			106/69	11	113/77	3	117/79	3			113/79	6	110/76	7	108/67	2
	P.R.	beats/min.			60	11	60	3	71	3			73	6	67	7	76	2
	UV	ml./min.			4.4	2	4.3	1	2.2	1			0.68	1	0.50	2	0.63	1
	C_{IN}	ml./min.			129	2	103	1	70	1			63	1	112	2	128	1
	C_{PAH}	ml./min.			553	2	474	1	347	1			301	1	544	2	714	1
	UNaV	mEq./min.			0.11	2	0.12	1	0.084	1			0.052	1	0.074	2	0.10	1
	UClV	mEq./min.			0.093	2	0.093	1	0.075	1			0.055	1	0.065	2	0.091	1
	UKV	mEq./min.			0.030	2	0.027	1	0.019	1			0.015	1	0.023	2	0.028	1
26 W. M. M 1.93	B.P.	mm. Hg			119/69	7	115/67	3	111/66	3			120/67	3	119/70	6	119/70	10
	P.R.	beats/min.			64	7	61	3	59	3			60	3	61	6	59	10
	UV	ml./min.			2.6	2	3.8	1	1.4	1			0.51	1	0.47	2	1.5	3
	C_{IN}	ml./min.			149	2	138	1	86	1			122	1	174	2	116	3
	C_{PAH}	ml./min.			703	2	632	1	377	1			467	1	731	2	665	3
	UNaV	mEq./min.			0.085	2	0.061	1	0.033	1			0.035	1	0.091	2	0.088	3
	UClV	mEq./min.			0.11	2	0.087	1	0.047	1			0.055	1	0.060	2	0.067	3
	UKV	mEq./min.			0.17	2	0.14	1	0.084	1			0.088	1	0.070	2	0.058	3
39 J. L. M 1.69	B.P.	mm. Hg			117/82	6	104/76	7					111/85	8	119/84	11	117/81	23
	P.R.	beats/min.			64	6	69	7					71	7	68	11	70	23
	UV	ml./min.			5.6	2	5.7	1					0.86	1	0.88	2	0.98	8
	C_{IN}	ml./min.			103	2	98	1					71	1	81	2	84	8
	C_{PAH}	ml./min.			540	2	512	1					402	1	442	2	522	8
	UNaV	mEq./min.			0.080	2	0.093	1					0.076	1	0.12	2	0.19	8
	UClV	mEq./min.			0.095	2	0.12	1					0.091	1	0.14	2	0.20	8
	UKV	mEq./min.			0.046	2	0.042	1					0.038	1	0.047	2	0.041	8

TABLE I-B

Hypertensive subjects—pre-operative

Patient Age and Sex Surface area (sq. m.)	Observations		Periods before congestion				Periods during congestion						Periods after congestion					
			Fourth and more		First through third		First		Second and third		Fourth and more		First		Second and third		Fourth and more	
	Function	Unit	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N
43 I. B. F 1.42	B.P.	mm. Hg			180/111	9	175/120	10	177/116	10			184/117	5	187/118	6	194/117	9
	P.R.	beats/min.			70	8	73	10	73	10			70	4	72	6	68	8
	UV	ml./min.			7.5	3	5.6	1	2.4	1			1.2	1	1.9	2	5.1	3
	C_{IN}	ml./min.			60	3	56	1	44	1			46	1	50	2	49	3
	C_{PAH}	ml./min.			346	3	342	1	255	1			310	1	329	2	330	3
	UNaV	mEq./min.			0.15	3	0.13	1	0.086	1			0.083	1	0.11	2	0.15	3
	UClV	mEq./min.			0.080	3	0.083	1	0.057	1			0.059	1	0.079	2	0.094	3
	UKV	mEq./min.			0.043	3	0.038	1	0.027	1			0.023	1	0.027	2	0.032	3
40 L. P. M 1.76	B.P.	mm. Hg			189/123	9	181/124	4					189/124	5	178/123	8	135/93*	23
	P.R.	beats/min.			75	9	74	4					78	5	78	8	78	23
	UV	ml./min.			10	2	12	1					11	1	8.3	2	0.38	5
	C_{IN}	ml./min.			91	2	83	1					78	1	67	2	64	5
	C_{PAH}	ml./min.			429	2	408	1					426	1	400	2	464	5
	UNaV	mEq./min.			0.13	2	0.13	1					0.11	1	0.091	2	0.022	5
	UClV	mEq./min.			0.15	2	0.16	1					0.12	1	0.11	2	0.033	5
	UKV	mEq./min.			0.040	2	0.057	1					0.039	1	0.039	2	0.038	5
43 K. W. M 1.84	B.P.	mm. Hg	191/121	10	181/119	11	131/90	13					153/103	6	122/84	11	159/106	18
	P.R.	beats/min.	74	10	73	11	60	11					68	6	66	11	71	18
	UV	ml./min.	1.1	3	1.02	3	0.59	1					0.75	1	0.48	2	0.84	3
	C_{IN}	ml./min.	97	3	95	3	59	1					111	1	94	2	113	3
	C_{PAH}	ml./min.	372	3	371	3	265	1					392	1	325	2	503	3
	UNaV	mEq./min.	0.11	3	0.092	3	0.066	1					0.034	1	0.021	2	0.046	3
	UClV	mEq./min.	0.12	3	0.10	3	0.071	1					0.047	1	0.047	1	0.054	3
	UKV	mEq./min.	0.096	3	0.093	3	0.066	1					0.16	1	0.11	2	0.18	3

* Patient had a spontaneous hypotensive episode of unexplained origin.

TABLE I-C

Hypertensive patients—postoperative

Patient Age and Sex Surface area (sq. m.)	Observations		Periods before congestion				Periods during congestion						Periods after congestion					
			Fourth and more		First through third		First		Second and third		Fourth and more		First		Second and third		Fourth and more	
	Function	Unit	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N
39 M R. S. 1.871	B.P.	mm. Hg			143/87	9	134/86	9	124/80	19	113/74	24	140/82	3	139/84	4	141/80	9
	P.R.	beats/min.			86	9	101	9	109	19	114	24	88	2	87	4	88	9
	UV	ml./min.			4.2	3	5.2	1	2.7	2	0.58	2	0.19	1	0.77	1	3.5	2
	C_{IN}	ml./min.			108	3	92	1	70	2	86	2	91	1	105	1	93	2
	C_{PAH}	ml./min.			521	3	465	1	347	2	399	2	423	1	530	1	503	2
	UNaV	mEq./min.			0.064	3	0.075	1	0.048	2	0.060	2	0.037	1	0.083	1	0.072	2
	UClV	mEq./min.			0.058	3	0.061	1	0.047	2	0.042	2	0.017	1	0.051	1	0.053	2
	UKV	mEq./min.			0.069	3	0.053	1	0.037	2	0.028	2	0.027	1	0.031	1	0.039	2
43 M J. O. 1.74	B.P.	mm. Hg			137/95	7	138/101	10	132/97	16			129/98	9	139/103	9	142/102	2
	P.R.	beats/min.			72	7	81	8	84	12			78	4	80	8	76	3
	UV	ml./min.			4.0	2	2.9	1	1.5	2			0.65	1	2.4	2	2.5	1
	C_{IN}	ml./min.			148	2	76	1	96	2			69	1	126	2	93	1
	C_{PAH}	ml./min.			554	2	343	1	436	2			347	1	609	2	470	1
	UNaV	mEq./min.			0.19	2	0.14	1	0.12	2			0.076	1	0.16	2	0.12	1
	UClV	mEq./min.			0.18	2	0.14	1	0.12	2			0.070	1	0.14	2	0.12	1
	UKV	mEq./min.			0.031	2	0.024	1	0.029	2			0.024	1	0.045	2	0.036	1
43 M K. W. 1.84	B.P.	mm. Hg			165/109	10	174/118	10	147/107	10			141/99	6	127/93	7	174/120	7
	P.R.	beats/min.			88	10	102	10	109	10			91	6	93	7	94	7
	UV	ml./min.			3.0	3	2.3	1	1.1	1			0.31	1	0.70	2	1.1	2
	C_{IN}	ml./min.			99	3	70	1	110	1			95	1	102	2	106	2
	C_{PAH}	ml./min.			482	3	306	1	416	1			374	1	516	2	466	2
	UNaV	mEq./min.			0.10	3	0.094	1	0.11	1			0.053	1	0.11	2	0.16	2
	UClV	mEq./min.			0.083	3	0.074	1	0.098	1			0.048	1	0.081	2	0.11	2
	UKV	mEq./min.			0.058	3	0.046	1	0.074	1			0.050	1	0.084	2	0.080	2

TABLE I-C—*Continued*

Patient Age and Sex Surface area (sq. m.)	Observations		Periods before congestion				Periods during congestion						Periods after congestion					
			Fourth and more		First through third		First		Second and third		Fourth and more		First		Second and third		Fourth and more	
	Function	Unit	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N
43 I. B. F 1.42	B.P.	mm. Hg			186/113	8	171/111	9	158/110	7			185/113	8	175/108	5	179/110	7
	P.R.	beats/min.			72	8	83	9	87	6			75	7	78	5	73	7
	UV	ml./min.			1.9	3	2.0	1	0.070	1			0.030	1	0.43	2	0.80	2
	C_{IN}	ml./min.			69	3	71	1	51	1			54	1	73	2	76	2
	C_{PAH}	ml./min.			341	3	345	1	247	1			270	1	361	2	374	2
	UNaV	mEq./min.			0.10	3	0.11	1	0.072	1			0.052	1	0.074	2	0.096	2
	UClV	mEq./min.			0.077	3	0.081	1	0.053	1			0.032	1	0.043	2	0.06	2
	UKV	mEq./min.			0.041	3	0.046	1	0.032	1			0.031	1	0.039	2	0.038	2
56 E. P. F 1.55	B.P.	mm. Hg			197/112	5	183/116	9	178/121	7			172/117	3	173/111	7	170/107	16
	P.R.	beats/min.			76	5	86	8	90	5			82	3	75	7	73	16
	UV	ml./min.			9.1	2	4.6	1	1.5	1			0.50	1	0.40	2	0.37	6
	C_{IN}	ml./min.			121	2	78	1	62	1			68	1	78	2	77	6
	C_{PAH}	ml./min.			663	2	407	1	348	1			387	1	450	2	447	6
	UNaV	mEq./min.			0.20	2	0.098	1	0.078	1			0.057	1	0.059	2	0.048	6
	UClV	mEq./min.			0.19	2	0.096	1	0.079	1			0.059	1	0.052	2	0.048	2
	UKV	mEq./min.			0.075	2	0.045	1	0.036	1			0.034	1	0.032	2	0.036	6
40 L. P. M 1.76	B.P.	mm. Hg			157/108	11	147/108	12					152/109	9	151/104	7	151/105	30
	P.R.	beats/min.			76	11	96	12					82	9	79	7	83	30
	UV	ml./min.			8.1	3	4.6	1					0.65	1	0.48	2	3.2	7
	C_{IN}	ml./min.			79	3	50	1					43	1	81	2	79	7
	C_{PAH}	ml./min.			502	3	360	1					300	1	521	2	593	7
	UNaV	mEq./min.			0.099	3	0.065	1					0.038	1	0.070	2	0.083	7
	UClV	mEq./min.			0.060	3	0.038	1					0.026	1	0.047	2	0.056	7
	UKV	mEq./min.			0.054	3	0.041	1					0.027	1	0.044	2	0.049	7

the true trends of the results.[10] It will be seen that in general the effects of venous congestion in the limbs upon renal clearances and the excretion of water and salt were essentially similar in the normotensive control subjects (Table I-A and Figures 1–4) and in the hypertensive patients both before (Table I-B) and after splanchnicectomy (Table I-C and Figures 5, 6). Thus, characteristically after about ten minutes of limb congestion, the urine flow began to decrease, frequently falling to one-tenth its previous rate. With the initial fall in urine flow there was a decrease in calculated *renal plasma flow* (PAH clearance) and *filtration rate* (inulin clearance) and in observed *sodium excretion, chloride excretion,* and sometimes *potassium excretion.* The percentage decrease in excretion of electrolytes and clearance substances was not so great as of water, so that the concentration of these materials in the urine usually rose, often to a marked degree (Figures 2, 4, 6).

Not only was the reduction of urine flow greater in amount, but it was also usually longer in duration than the other renal changes produced by venous congestion of the limbs. Thus, during the first part of the long (40- to 60-minute) congestion experiments (Figures 1, 2, 5, 6) the urine flow decreased, and it usually remained at the lower levels, even during the last 20 to 30 minutes of congestion when the calculated renal plasma flow and glomerular filtration rate (and occasionally the electrolyte excretion) were often returning towards if not to control levels. Sodium, chloride, and potassium excretion usually remained definitely below control

[10] Thus the control period *immediately* before congestion of the limbs (first period before) has been averaged together with the two prior periods under "First through third." When more than three control periods were used all the earlier periods have been averaged under "Fourth and more." The periods during congestion and after its release have also been averaged together in a similar way, except for the first period in each case, which has been recorded separately as probably not indicative of the subsequent general trend. Likewise, in the statistical analysis in Table II comparisons have been made only between those portions of the experiments which are representative of a relatively "steady state."

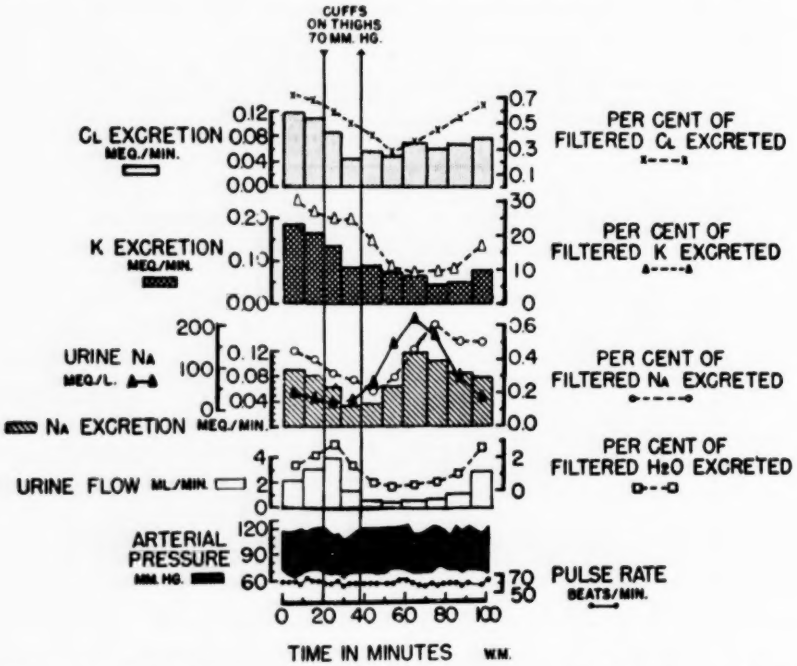

FIG. 4. CHART OF ELECTROLYTE AND WATER EXCRETION DURING THE EXPERIMENT SHOWN IN FIGURE 3

Method of charting as in Figure 2.

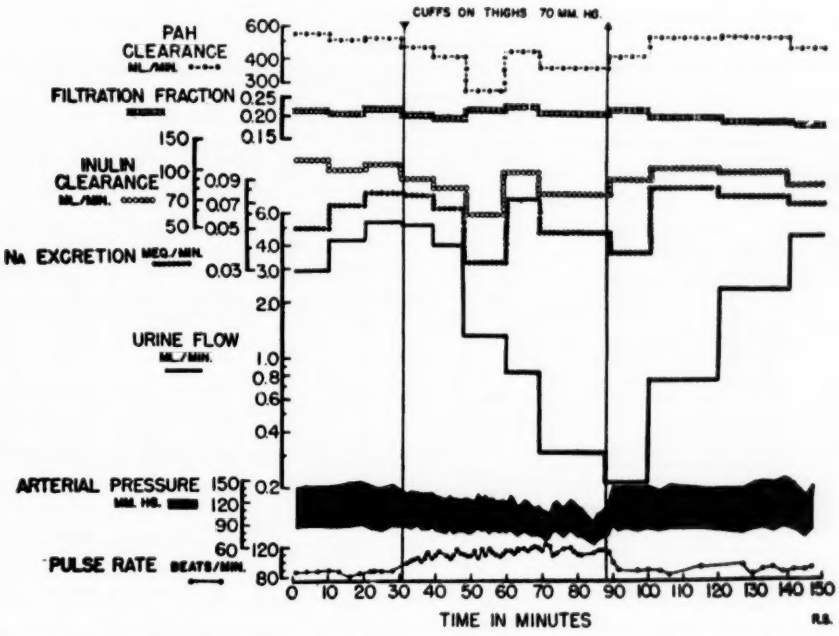

FIG. 5. CHART OF AN EXPERIMENT ON A HYPERTENSIVE SUBJECT TWO WEEKS AFTER A LUMBODORSAL SYMPATHECTOMY CONDUCTED SIMILARLY TO THAT SHOWN IN FIGURE 1

Method of charting as in Figure 1.

levels throughout the congestion period. On release of the congesting cuffs there was often a further slight to moderate decrease in urine volume and electrolyte excretion for one or two periods, then a gradual return to control levels, the urine flow usually returning more slowly than the electrolyte excretion.

During short (10- to 30-minute) periods of venous congestion similar effects occurred (Figures 3 and 4), although usually not to the same degree as during the longer periods, so that by the time the congesting cuffs were released the urine flow and other renal functions, though decreased, had usually not fallen to their minimum values. After release of the cuffs these functions continued to fall for one or two periods; indeed, it appeared in many of the shorter tests as if the release of the congesting cuffs was a brief stimulus to further small reductions in urine flow, renal clearances, and sodium excretion. Even including these reductions, the total decreases in sodium excretion as a rule were not as great as from the long periods of congestion, although the decreases in urine flow were about as great. After release of the cuffs the urine flow often returned more slowly than the other functions, whereas renal plasma flow, glomerular filtration rate and sodium and chloride excretion tended to increase promptly together. Potassium excretion behaved more irregularly, but usually fell during congestion and returned to control levels after release but not necessarily simultaneously with sodium and chloride excretion.

General Hemodynamic Effects

As a rule *pulse rate* increased moderately during the period of venous congestion, while systolic and diastolic arterial pressures were not greatly changed. Sometimes, however, pulse rate rose markedly and occasionally arterial pressure fell (Figure 5), rarely even to collapse levels. When a definite hypotensive reaction occurred it was always followed by very marked responses of the same general type as already described, the antidiuresis being particularly profound and sometimes persisting for more than an hour after release of

TABLE II

Statistical analysis

		Systolic pressure *mm. Hg*	Diastolic pressure *mm. Hg*	Pulse rate *beats/min.*	UV *ml./min.*	C_{IN} *ml./min.*	C_{PAH} *ml./min.*	UNaV *mEq./min.*	UClV *mEq./min.*	UKV *mEq./min.*
Periods before congestion First through third (Control)	Mean (n = 18)	132	85	77	5.1	107	554	0.14	0.10	0.051
	Standard error of mean	7	4	3	0.6	6	30	0.013	0.012	0.008
Periods during congestion Second and third	Mean (n = 18)	126	87	87	2.0	71	360	0.088	0.067	0.032
	Standard error of mean	6	4	4	0.3	5	22	0.008	0.007	0.004
	Mean difference from control	−6	3	10	−3.0	−36	−194	−0.050	−0.036	−0.018
	Standard error of difference	2	1	2	0.6	5	26	0.007	0.008	0.005
	Significance of difference (P)	0.03	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fourth and more	Mean (n = 6)	114	81	100	0.6	80	382	0.052	0.036	0.022
	Standard error of mean	2	2	7	0.2	5	30	0.008	0.008	0.001
	Mean difference from control (n = 6)	−5	3	18	−4.9	−24	−179	−0.064	−0.044	−0.022
	Standard error of difference	6	3	4	1.0	9	43	0.029	0.016	0.006
	Significance of difference (P)	0.46	0.39	<0.01	<0.01	0.05	<0.01	0.08	0.04	0.02
Periods after congestion Fourth and more	Mean (n = 15)	137	89	81	2.8	96	492	0.11	0.082	0.038
	Standard error of mean	7	4	4	0.6	5	30	0.012	0.009	0.004
	Mean difference from congestion periods second and third (n = 15)	8	−1	−9	1.1	25	142	0.026	0.014	0.005
	Standard error of difference	3	2	2	0.5	5	30	0.008	0.006	0.002
	Significance of difference (P)	<0.01	0.56	<0.01	0.05	<0.01	<0.01	<0.01	0.03	0.08
	Mean difference from congestion periods fourth and more (n = 5)	9	−2	−14	3.2	9	107	0.026	0.021	0.011
	Standard error of difference	5	2	4	0.9	4	40	0.008	0.007	0.002
	Significance of difference (P)	0.14	0.37	0.02	0.02	0.08	0.06	0.03	0.04	<0.01
	Mean difference from control (n = 15)	1	2	2	−2.0	−11	−41	−0.028	−0.026	−0.015
	Standard error of difference	3	1	1	0.8	5	22	0.016	0.012	0.008
	Significance of difference (P)	0.76	0.18	0.11	0.03	0.06	0.09	0.11	0.05	0.08

the cuffs, whereas the arterial pressure usually returned promptly to control levels.

Hypertensive patients, though usually more unstable in their blood pressure reactions, showed no striking differences after as compared with before lumbodorsal splanchnicectomy, or as compared with the more normal control subjects. During venous congestion of the limbs the changes in arterial pressure, pulse rate, urine flow and renal clearances were generally similar to those already described in the normal subjects.

SPECIAL RESULTS

I. *Hematologic Effects*

During and after venous congestion of the limbs there was, if anything, a tendency for the arterial blood hematocrit and protein content to rise, particularly immediately following the release of the congesting cuffs.

II. *Effects of Sensory Stimulation and Discomfort*

Because pain and even moderate discomfort are known to be potent antidiuretic stimuli in animals, care was taken to avoid such factors as much as possible in the course of the tests described above. In addition, some experiments were done deliberately (as mentioned under "Methods") to determine the role of these factors. Thus, it was found that inflation of the congesting cuffs for only 30 seconds at five-minute intervals produced no appreciable effects upon the hemodynamic or renal functions measured. Furthermore, continuous *compression* of the entire limbs of a similar degree and duration as the congestion used at a different time caused no consistent effects on the measurements made. There was often a moderate *increase* in diuresis during such compression, although occasionally there was a brief, slight decrease. However, patients uniformly reported this procedure to be distinctly more uncomfortable than congestion of the limbs at the same pressure.

III. *Effects of Emotion*

Occasionally, particularly at the end of long experiments, patients became fatigued and emotionally upset. It was interesting, in view of the possible influences of this reaction upon renal and circulatory measurements being made, that it had no consistent effects. Indeed, when venous congestion was applied during such a reaction, patients as often as not failed to have the antidiuresis and

associated renal changes that they characteristically had when under no discernible emotional stress. On the other hand, occasional patients (*e.g.*, patient R. F.) failed to show the characteristic reactions early in a test, although they did later. In these cases it usually appeared that there had been either an inadequate rest period for hemodynamic and body fluid adjustments to the experimental condition (resting horizontal position) to come into equilibrium; or a preliminary over-hydration with ingestion of a large amount of fluid prior to the test.

IV. *Effects of Venous Congestion of Other Areas or at Other Pressures*

A number of incomplete "pilot" experiments were done in which the arms were congested instead of the legs or in which either area was congested at 30 instead of 70 mm. Hg. In general, the effects of congesting the arms or of using lower congesting pressures on the thighs upon water and electrolyte excretion were the same qualitatively though smaller quantitatively, as compared with the effects of congesting the legs at higher pressures.

V. *Effects of Urine Volume*

Early in this work it became apparent that the most marked if not the most consistent renal change produced by venous congestion of limbs in a patient under diuresis was antidiuresis. Indeed, it was considered possible that this was the only primary renal change effected by venous congestion and that other apparent changes were either secondary to this or were even artefacts due to the use of methods dependent upon adequate urine flow. Attempts were made to rule out these possibilities by careful recalculation of the data so as to compare the average clearances per minute for all the periods combined during the entire antidiuretic response (from full diuresis back to full diuresis), with the average of the combined control values before and after the antidiuresis. The average clearances for these combined periods in nearly every case clearly indicated reductions of renal plasma flow and glomerular filtration rate

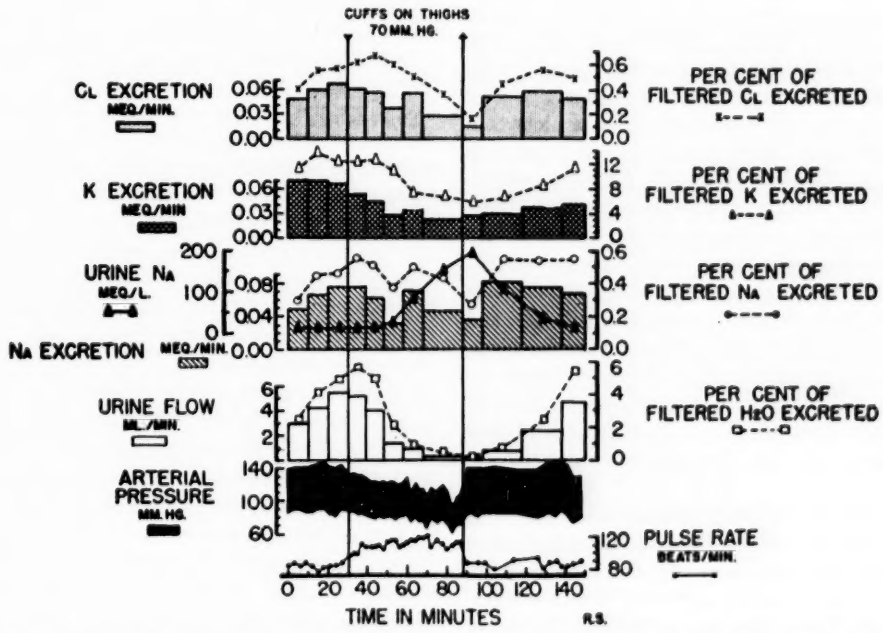
CUFFS ON THIGHS 70 MM. HG.
Cl EXCRETION MEQ/MIN.
K EXCRETION MEQ/MIN
URINE Na MEQ/L.
Na EXCRETION MEQ./MIN.
URINE FLOW ML./MIN.
ARTERIAL PRESSURE MM. HG.
PER CENT OF FILTERED Cl EXCRETED
PER CENT OF FILTERED K EXCRETED
PER CENT OF FILTERED Na EXCRETED
PER CENT OF FILTERED H2O EXCRETED
PULSE RATE BEATS/MIN.
TIME IN MINUTES
R.S.

FIG. 6. CHART OF ELECTROLYTE AND WATER EXCRETION DURING THE EXPERIMENT SHOWN IN FIGURE 5
Method of charting as in Figure 2.

during the antidiuretic response, but these were of a degree ordinarily considered insignificant for single short periods. It was therefore judged impossible to decide conclusively by this method how greatly "dead space" and "washout" errors contributed to the apparent results, although in certain patients in whom obvious overshoots in clearance occurred on reappearance of diuresis (*e.g.*, R. P., R. F., L. B., W. M., G. V., R. S., and J. O.) this seemed quite substantial. In other patients, however, the changes in clearances and electrolyte excretion bore no (or even an opposite) relationship to urine flow. For example, in patient M. C. urine flow remained low after venous congestion was released whereas the clearance of inulin and PAH returned to control values. The same was true in patient J. B., although in this patient sodium excretion also returned to control values, whereas in M. C. it remained low with the urine flow. Further, sodium excretion in occasional patients (G. V. and J. L.) failed to be lowered significantly, although UV, C_{IN} and C_{PAH} decreased in the usual manner.

Another type of recalculation of the data was made in an attempt to shed light on these points. The average percentage change (from control) in each function (clearance) during each period was calculated for the entire normotensive, and the entire postoperative hypertensive groups, and plotted as two (average) experiments. These plots confirmed the impressions already gained from plots of separate experiments, namely that although initially (in the early congestion periods) all the functions decreased sharply together, later on (4th through 6th periods) the urine flow (water excretion) was decreased most, sodium and chloride less (and about equally), potassium still less, and PAH (plasma flow) and inulin (glomerular filtration rate) least, but nevertheless were definitely decreased. Furthermore, the responses of the normotensive subjects were not significantly different from those of the postoperative hypertensive patients.

These results suggested that although the urine volume and the clearances of inulin, para-aminohippurate, and sodium usually decreased and increased together during and after venous congestion of the limbs, they did not necessarily do so equally and that although the changes in urine volume might magnify the apparent changes in clearances, the latter appeared to be essentially independent effects of the venous congestion and its release. These opinions were confirmed definitely as regards sodium excretion and tentatively as regards renal plasma flow and glomerular filtration rate in subsequent experiments in patients with diabetes insipidus and in normal subjects under mannitol diuresis (6, 7). In these experiments there were minimal changes in urine volume during venous congestion of the limbs, although there were the usual changes in sodium excretion and similar (though less marked) changes in clearance of inulin and PAH.

VI. *Effect of Changes in Glomerular Filtration Rate (Inulin Clearance)*

Additional evidence that the apparent changes in electrolyte excretion during and after venous congestion were not entirely artefacts due to collection errors but were, in part at least, the result of real differences (whether primary or secondary) in the treatment by the tubules of the filtrate presented to them was shown by calculating the "Tubular rejection fraction" or "Percentage of filtered substance excreted" (E/F) (Figures 2, 4, 6). (Space does not permit inclusion of these data in the tables, but their nature can be determined by calculating the ratio, UV times Na, K or Cl, over C_{IN}, since the plasma levels of these electrolytes showed no significant changes during the experiments.) The calculations usually showed the percentage of the filtered water, sodium, chloride and, to a lesser degree, potassium which was actually excreted in the urine to be decreased during venous congestion and immediately after its release, and at times not to return to control levels until after reductions in glomerular filtration rate (inulin clearance) had disappeared. However, there was no uniform pattern in the behavior of sodium E/F. Thus, during and after the shorter congestion experiments the decreases in E/F of sodium were generally less than during and after the longer congestion periods, although the other results might be comparable. Therefore, in the shorter experiments it appeared that the excretion of sodium bore a fairly "passive" or proportional relationship to glomerular filtration rate. However, in the longer experiments sodium excretion appeared to take a more independent course, resulting in greater

changes in E/F. Thus, patients M. C. and R. P. showed further decreases in the excretion and E/F of sodium at the end of the congestion period when glomerular filtration rate was increasing, while patient W. Mc. showed an unchanged excretion with a decreased E/F at that time. In patient L. B., on the other hand, the changes in glomerular filtration rate were never great, while the typical decreases in the excretion and E/F of sodium occurred.

Potassium excretion (and E/F) in the long and short congestion experiments tended to behave similarly, the excretion varying directly with the glomerular filtration rate and E/F showing minor though fairly consistent decreases.

DISCUSSION

The afferent and efferent mechanisms involved in the renal responses to venous congestion of the limbs reported in this paper are questions of considerable interest. Of greatest interest perhaps is the nature of the stimulus or stimuli calling forth these responses. Since the subjects remained in the supine position throughout the experiments postural stimuli can be eliminated. Of the various remaining possibilities, a reduction in effective circulating blood volume seems one of the more likely. Decreases in urine flow and sodium excretion would appear logically to be homeostatic responses tending to offset any reduction in effective blood volume. Although these responses were most profound when there was collapse of the arterial pressure they could occur before there was any appreciable change in pressure. However, this does not imply that therefore they must have been responses to some other stimulus, since, as previous studies in this laboratory have shown, venous congestion of the limbs always tends to cause arterial pressure to decrease, and does cause it to decrease if the counteracting vasomotor adjustments are blocked (8). It is, therefore, reasonable to suppose that the renal responses to venous congestion of the limbs may be integral parts of the general homeostatic mechanisms for counteracting the hypotensive effects of an inadequate circulating blood volume.

There has been considerable speculation upon the possibility that a decrease in effective circulating blood volume stimulates "volume receptors" to cause salt and water retention of the type described here. If the term "volume receptor" is meant to connote end-organs sensitive to changes in volume occurring without concomitant changes in pressure, the concept seems totally unrealistic. There are no known sensory end-organs of this type, and indeed it is theoretically impossible to have passive changes in volume of even inelastic vessels that are not associated with at least small changes in pressure. If, on the other hand, "volume receptor" implies that the changes in volume are relatively large as compared with the changes in pressure, the term is acceptable, but seems to add little to our thinking about the problem.

It would appear more fruitful to inquire what and where the changes in pressure may be that could promote reduction in salt or water excretion. Changes in venous, right atrial, right ventricular or pulmonary arterial pressures have all been considered as stimuli possibly capable of producing the renal responses reported in this paper. Studies designed to throw light on these possibilities have been made and have been or will be reported (9, 10, 11). Suffice it to say here, they do not appear to be very likely possibilities. Neither do stimuli such as discomfort, distention of the veins, loss of fluid into or stretching of the tissues locally in the experimental limbs appear to be responsible (10).

As to the effector mechanisms of the renal responses to venous congestion of the limbs, it appears that they are not dependent upon the splanchnic sympathetic nervous system. As shown in this paper, the responses occur similarly in splanchnicectomized hypertensive patients as in normotensive or unoperated hypertensive subjects. Presumably, therefore, they are mediated at least in part through humoral or local renal mechanisms. The most obvious of the humoral mechanisms that may be considered are the posterior pituitary antidiuretic hormone effects on urine flow, and the adrenal cortical hormone effects on sodium excretion. Studies designed to explore these and other humoral possibilities have also been carried out and will be reported (6, 7).

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THE EFFECTS OF ANTIMICROBIAL AGENTS UPON CHOLINE DEGRADATION IN THE INTESTINAL TRACT

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INTRODUCTION

A variety of intestinal bacteria have been shown to be capable of degrading choline to trimethylamine (1–3) which is readily absorbed from the intestine and excreted in the urine mainly as trimethylamine oxide. When a relatively large amount of choline is ingested by normal persons, about 60 per cent appears in the urine as total trimethylamines (TTMA), mostly within 24 hours (3).

Part of the evidence gathered to establish this phenomenon was that, following ingestion of choline with aureomycin, much less TTMA than usual were excreted (3, 4). Further experiments were undertaken to elaborate upon the inhibition of TTMA formation from choline by antimicrobial agents.

Several antimicrobial agents were administered in single therapeutic doses simultaneously with choline to determine whether they exhibited an effect similar to aureomycin. The period of suppression of bacterial degradation of choline was explored by allowing a time interval between the ingestion of the antibiotics and the choline. The development of bacterial resistance to the suppressive effect was studied by the protracted administration of an antibiotic in conjunction with repeated ingestion of choline.

MATERIAL AND METHODS

Of the 36 subjects used in this study, 26 were patients hospitalized at Cook County Hospital for a variety of causes other than intestinal or hepatic disease, and 10 were young, healthy physicians.

Choline was given orally in the form of bicarbonate [1] in amounts containing 4 Gm. choline base (460 mg. N) with the exception of the four normal subjects who received 5.4 Gm. choline chloride (540 mg. N). This is twice the amount of choline used in previous studies (3). This large dose of choline was chosen since the use of a larger dose would give a better indication of the depression of trimethylamine formation by the antimicrobial agents. Following this, the urine was collected for the first and second 24 hours in separate containers using hydrochloric acid as preservative. The 24 to 48 hour excretions were determined to serve as a check that no unusual delay in TTMA excretion occurred, which might account for low values in the first 24 hours. Urinary TTMA were determined by the reduction-aeration-reineckate method reported previously (3) and the results are expressed in mg. TTMA N. At least one week was allowed to elapse between the first (control) determination and the one in which an antimicrobial agent was given together with choline. This interval was chosen to make certain that the TTMA excretion had returned to the small basal level (5 to 15 mg. TTMA N per 24 hours), resulting probably from the degradation of dietary choline. In the four instances in which one subject was used for two tests, at least two weeks elapsed between determinations.

In the single experiments, the following antimicrobial agents were given orally: aureomycin (500 mg.); terramycin (500 mg.); penicillin O (600,000 units); dibenzylethylenediamine penicillin (DBED-penicillin) (600,000 and 25,000 units); sulfaphthalidine (4 Gm.); and gantrisin (4 Gm.). In the protracted experiments, DBED-penicillin was given in amounts of 200,000 units with choline and, on the days in between, 100,000 units were given twice daily. In similar experiments one Gm. of aureomycin was given daily for seven days. Aureomycin was also given intravenously in the form of 500 mg. in saline the evening before the administration and the same dose the next morning when the test dose of choline was given orally.

From terramycin, penicillin O, DBED-penicillin, sulfaphthalidine and gantrisin, no trimethylamine was recovered *in vitro* when the method used in the urines was applied. Aureomycin produced *in vitro* traces of trimethylamine, but failed to produce any increase in urinary TTMA excretion after oral administration in a normal person.

RESULTS

The 32 subjects, who received 4 Gm. choline, excreted from 220 to 315 mg. TTMA N within the first 24 hours after ingestion of the choline, with a mean excretion of 262 mg. Only in three instances

[1] Thanks are due to Commercial Solvents Corporation for the generous supply of choline bicarbonate syrup.

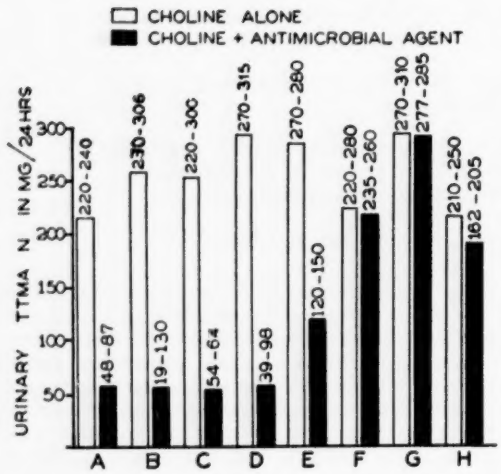

FIG. 1. URINARY EXCRETION OF TOTAL TRIMETHYLAMINES AFTER THE INTAKE OF 4 GM. CHOLINE BASE (460 MG. N) WITH AND WITHOUT SIMULTANEOUS ADMINISTRATION OF AN ANTI-MICROBIAL AGENT

A. Oral, 500 mg. aureomycin.
B. Oral, 500 mg. terramycin.
C. Oral, 600,000 units penicillin O.
D. Oral, 600,000 units DBED-penicillin.
E. Oral, 25,000 units DBED-penicillin.
F. Oral, 4 Gm. sulfaphthalidine.
G. Oral, 4 Gm. gantrisin.
H. Intravenous, 1 Gm. aureomycin.

of the entire study was the 24 to 48 hours excretion significant. The average urinary excretion of TTMA N in this period was 34 mg. (about 7 per cent of the 460 mg. of choline N fed) for the controls and tests. Since one-third to one-half of this amount is basal excretion, only 3 to 5 per cent of the choline nitrogen fed was excreted in the second 24 hours urine collection, and this small amount has been disregarded in the consideration of the results.

Effect of antimicrobial agents fed simultaneously with choline

Three persons, given aureomycin together with choline, excreted an average of 68 mg. of TTMA N compared to 233 mg. when choline was fed alone (Figure 1A). Following administration of terramycin to three subjects, the mean TTMA excretion was 64 mg. N in contrast to 270 mg. N after feeding choline alone (Figure 1B). In the two subjects, each of whom received penicillin O together with choline, the mean control TTMA N excretion of 260 mg. was reduced to 59 mg. (Figure 1C). DBED-penicillin, given to seven subjects in doses of 600,000 units together with choline, reduced the TTMA excretion from an average of 286 mg. to 65 mg. N (Figure 1D). Following the administration of 25,000 units of DBED-penicillin to two subjects, the mean TTMA N excretion of 280 mg. was reduced to 136 mg. (Figure 1E). Sulfaphthalidine (Figure 1F) and gantrisin (Figure 1G), both given in amounts of 4 Gm. to three patients, did not significantly alter the average TTMA excretion following ingestion of choline. Intravenous aureomycin also had hardly any effect in three subjects (Figure 1H).

Five subjects received 600,000 units of DBED-penicillin 24 hours before the standard dose of choline. Urine collection was begun with the ingestion of the choline. The average control TTMA N excretion was 265 mg. whereas after penicillin administration the excretion was 210 mg. (Figure 2).

Prolonged administration of antibiotic agent

In five persons the TTMA excretion, after administration of choline alone and of choline with 200,000 units DBED-penicillin, was determined.

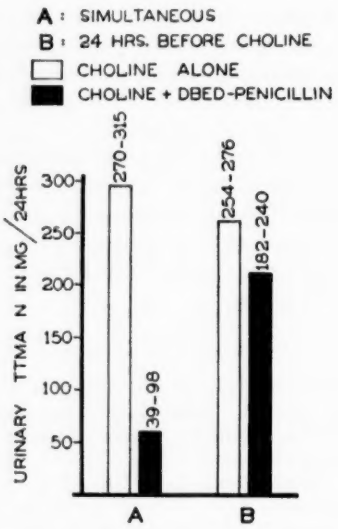

FIG. 2. MEAN URINARY TOTAL TRIMETHYLAMINES OF THREE SUBJECTS AFTER ADMINISTRATION OF 4 GM. CHOLINE BASE (460 MG. N) ALONE, SIMULTANEOUSLY OR 24 HOURS AFTER ADMINISTRATION OF 600,000 UNITS OF DBED-PENICILLIN

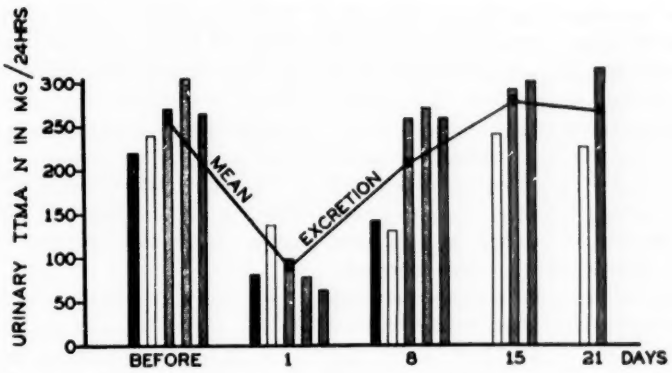

FIG. 3. URINARY TOTAL TRIMETHYLAMINES EXCRETION OF FIVE SUBJECTS FOLLOWING THE INTAKE OF 4 GM. OF CHOLINE (460 MG. N) BEFORE AND DURING DAILY ADMINISTRATION OF 200,000 UNITS OF DBED-PENICILLIN FOR SEVERAL WEEKS

Subsequently, they were fed daily doses of 100,000 units twice a day of DBED-penicillin for one to three weeks. At weekly intervals, a standard dose of choline was given and the urinary TTMA were determined. The penicillin administration was not interrupted during the period of the tests with choline. In these persons, the mean control TTMA N excretion was 263 mg. The excretion after the initial dose of penicillin was significantly reduced (89 mg. N); after the first week of daily administration of penicillin an almost complete return (220 mg. N) to the control value was observed. In the three persons who were continued on penicillin for two weeks, and in the two who were continued on penicillin for three weeks, the values had returned to control levels (278 and 270 mg. TTMA N, respectively) (Figure 3).

Four normal subjects were given aureomycin, one Gm. a day continuously for seven days. Choline chloride, 5.4 Gm. a day, was given on the first, the second, and the seventh day. A decrease of urinary TTMA excretion of approximately 50 per cent of the control level was noted following administration of aureomycin for one day, but the TTMA excretion returned to control levels by the seventh day.

DISCUSSION

The antibiotic agents studied are capable of inhibiting significantly the degradation of choline to trimethylamine by intestinal bacteria. This is concluded from the reduction in urinary excretion of TTMA from about 50 to 60 per cent of the choline N fed to only 12 to 16 per cent following the oral ingestion of aureomycin, terramycin, penicillin O, and DBED-penicillin. This conclusion is based on the previous observations that bacteria did not degrade choline to any other nitrogenous compound other than trimethylamine, that ingested trimethylamine is quantitatively excreted in the urine, and that, in conditions with reduced TTMA excretion, no appreciable amount of choline appeared in the feces. Since the reported effect is produced by antimicrobial agents of different chemical constitution, a chemical action can be excluded. The fact that soluble penicillin O is equally effective in the doses used, as the slightly water-soluble DBED-penicillin, suggests that the inhibition occurs in the upper portion of the small intestine before adsorption of the antibiotic takes place. When aureomycin is given intravenously a small amount is excreted in the bile (5, 6) and inhibition of colonic bacteria has been found. Apparently, concentrations sufficient for the purpose of this experiment were not reached, in the time required by this route, that would exert an inhibitory effect. The sulfa drugs tested were ineffective. This observation is surprising in view of the known inhibitory effect of sulfonamides; however, sulfonamides and antibiotics act through different pathways.

The suppressive effect of antibiotics is of short duration. When penicillin in effective doses is given 24 hours before choline, TTMA are ex-

creted in similar amounts as in the controls, suggesting bacterial recovery within 24 hours. That the inhibitory effect is not continuously exerted is shown by daily feeding of penicillin. The inhibitory effect is demonstrable after four days (3, 4). However, within one week, considerable recovery of TTMA excretion occurs, and after two and three weeks of feeding penicillin or aureomycin daily, control amounts of TTMA are excreted after a standard dose of choline. This represents an analogy to the transient effect of antimicrobial agents in improving hepatic coma, an effect which has been noted by some investigators (7, 8), and which is lost within a short period. It may well be that toxic amines, possibly responsible for hepatic coma, are formed in the intestine from amino acids, just as the pharmacologically inert trimethylamine is formed, and the antimicrobial agents may temporarily inhibit their formation. After the bacteria become refractory, coma reappears if the hepatic condition has not improved sufficiently in the interim.

At present, it cannot be decided whether the discussed antibiotic effect upon the intestinal choline degradation is of physiological significance. Aureomycin, terramycin, and penicillin have shown a definite delaying effect on experimental dietary necrosis and cirrhosis (9, 10, 11). In the short-term experiments, dealing with fatty liver and hemorrhagic cortical necrosis of the kidney, the beneficial effect of antibiotics may be related to the inhibition of intestinal degradation of choline (12). On the other hand, it is difficult to explain the prolonged effect of antibiotics, such as aureomycin and penicillin, on experimental dietary cirrhosis and necrosis of the liver, when one considers their apparently only transient effect on the intestinal flora, as exemplified by the intestinal degradation of choline.

SUMMARY

Oral aureomycin, terramycin, penicillin O, and dibenzylethylenediamine penicillin suppress the urinary trimethylamines excretion after simultaneous ingestion of a test dose of choline. In view of previous observations, this can be considered a suppression of intestinal degradation of choline to trimethylamine by intestinal bacteria.

Intravenous aureomycin and oral sulfaphthalidine and gantrisin are ineffective.

The described antimicrobial effect disappears within 24 hours after the administration of a single dose of penicillin. With continuous administration of pencillin, the antimicrobial effect disappears within one to two weeks indicating refractiveness of the bacteria.

The significance of these findings and the understanding of the effects of antibiotics in hepatic coma and choline metabolism are discussed.

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EFFECT OF PHENYLBUTAZONE (3,5 DIOXO-1,2-DIPHENYL-4-N-BUTYLPYRAZOLIDINE) ON RENAL CLEARANCE OF URATE AND OTHER DISCRETE RENAL FUNCTIONS IN GOUTY SUBJECTS [1]

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Phenylbutazone is a potent analgesic agent currently employed in the treatment of various arthritic disorders (1) including acute gouty arthritis (1, 2). It is generally agreed that administration of the drug to gouty subjects effects a sharp lowering of the serum urate level (1, 2). The mechanism by which this is accomplished is still obscure.

Evidence that phenylbutazone is a uricosuric drug which depresses tubular reabsorption of urate has been presented elsewhere (2, 3). Further data are recorded here. These deal in greater detail with a) the acute and protracted effects of intravenous and oral phenylbutazone administration upon the renal clearance of urate and upon serum urate concentration; b) the effects of intravenously administered phenylbutazone upon the renal clearances of inulin, endogenous creatinine and PAH, the urinary excretion of sodium, potassium, chloride and inorganic phosphate, and upon Tm_{PAH}; and c) the effects of simultaneous administration of phenylbutazone and probenecid (Benemid) upon urinary urate excretion.

METHODS

The acute effects of intravenously administered phenylbutazone on the simultaneous renal clearances of urate, inulin, endogenous creatinine and p-aminohippurate, using techniques previously described (4), were studied in ten gouty subjects in the intercritical phase of the disease. All abstained from meat and purine-rich food for three days prior to the study, which was started in the morning during the post-absorptive state. Liberal quantities of water were administered orally to maintain urine flows higher than 3 cc. per min. throughout the course of the acute clearance determinations. Following three 10 to 15 minute control urine collection periods, 1 or 2 Gm. of phenylbutazone (12 to 27 mg. per Kg. body weight) as the sodium salt in 20 per cent solution, further diluted in 100 cc. of normal saline solution, were administered intravenously at a rate of approximately 5 cc. per min. After the drug had been infused the bladder was washed with distilled water and urine was collected for six to eight 15 to 20 minute periods. Urine collections were continued every two hours during the day and every four hours during the night for an additional 18 to 20 hours in order to follow endogenous creatinine and urate clearances.

In two of the subjects, simultaneous inulin, p-aminohippurate and urate clearances were determined during slow phenylbutazone infusions so as to correlate the effects with rising plasma phenylbutazone concentrations. In three additional subjects this correlation was studied following rapid infusion of the drug. In two of the subjects the effect of phenylbutazone upon Tm_{PAH} was studied using standard techniques (5).

The effects of intravenous administration of phenylbutazone on the renal excretion of sodium, potassium, chloride, inorganic phosphate and urate, and on the endogenous creatinine clearance, were studied in three hospitalized gouty subjects during a three-day control period, for two to four days of phenylbutazone administration, and three days thereafter. These subjects were maintained on a constant weighed diet and constant salt and water intake. The daily caloric intake was 1800 for two of the subjects and 1600 for the third. The protein intake was 60 Gm. per day for all, and the sodium chloride intake 3.5 Gm. per day for two, and 2.5 Gm. per day for one. Urine was obtained by spontaneous voiding, the collection periods being 9 a.m. to 3 p.m., 3 p.m. to 9 p.m. and 9 p.m. to 9 a.m. After three control period days 0.8 Gm. of phenylbutazone was administered intravenously in 0.2 per cent solution made up in 5 per cent glucose; this was delivered in divided doses of 0.2 Gm. each at 9 a.m. and 3 p.m. and 0.4 Gm. at 9 p.m. Blood samples were obtained just prior to each infusion.

The effect of orally administered phenylbutazone on the daily urinary output of urate was also studied in eight ambulatory subjects pursuing their normal daily activities. They received a 60 to 70 Gm. protein diet for 3 to 10 days preceding the control urine collections. After three 24-hour control urines and one or two control plasma urate determinations were obtained, 0.8 Gm. of phenylbutazone was administered daily in four divided doses for four to

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seven days. Plasma urate concentrations were determined periodically and urinary urate excretion measured daily throughout the period of study. In one of the subjects, T-1824 spaces were measured before and after 24, 48 and 96 hours of drug administration.

The effect of simultaneous administration of phenylbutazone and probenecid upon urinary urate excretion was studied in seven subjects.

The analytical methods for inulin, PAH, creatinine, sodium, potassium, chloride, inorganic phosphate and urate determinations in plasma and urine were those previously employed in the corresponding probenecid study (4). All blood samples were heparinized and the analyses were performed on plasma. In order to ascertain whether phenylbutazone interfered with the method employed for urate determination in plasma and urine, the following studies were performed: 1) Serial dilutions of phenylbutazone (10 to 40 mg. per cc.) were added to standard urate solutions and to urine; 2) varying proportions of urine from a subject taking phenylbutazone were added to standard urate solutions and to urine from a subject not taking phenylbutazone; 3) equal portions of plasma from a subject receiving phenylbutazone were mixed with plasma from a subject not receiving the drug; 4) all of the mixtures represented in 1, 2, and 3 were subjected to uricase digestion. In none of these studies was there evidence of interference with the recoveries of urate.

Plasma phenylbutazone concentrations were determined

TABLE I

Acute effects of intravenous administration of phenylbutazone (PB) on renal clearances of urate, inulin and PAH in ten subjects with non-tophaceous gout

(Patients listed in descending order of drug dosage/Kg. body weight)

Subject (Age)	Period	P_{urate}	Renal clearances			Clearance ratios	
			C_{urate}	C_{In}	C_{PAH}	$C_{urate}/C_{In} \times 100$	C_{In}/C_{PAH}
	min.	*mg. %*	*cc./min.*	*cc./min.*	*cc./min.*		
L. W.	Control	8.3	4.22	105	449	4.03	0.234
44	PB 2.0 Gm. I.V. (27 mg./Kg.)						
	15	8.1	16.3	97.4	457	16.7	0.214
	30	7.6	*37.6**	104	*364*	*36.2*	0.286
	45	7.4	19.7	102	401	19.3	0.255
	60	7.2	17.3	107	395	16.2	0.271
	75	7.2	16.7	106	392	15.8	0.271
	105	7.2	14.1	105	395	13.4	0.266
	135	7.2	13.1	110	407	11.9	0.270
S. H.	Control	8.4	6.54	96.6	456	6.80	0.212
45	PB 2.0 Gm. I.V. (26 mg./Kg.)						
	15	7.6	*33.6*	88.1	384	*38.1*	0.230
	30	7.6	20.3	95.2	492	21.3	0.194
	50	7.6	16.8	95.2	462	17.7	0.206
	65	7.6	17.1	84.0	363	20.4	0.232
	95	7.4	19.7	77.6	341	25.4	0.228
	125	7.1	17.1	70.6	*299*	24.2	0.236
M. S.	Control	8.6	7.12	85.2		8.40	
57	PB 1.8 Gm. I.V. (24 mg./Kg.)						
	40	8.3	8.03	88.4		9.10	
	100	7.8	*15.3*	74.1		*20.6*	
	120	7.6	15.0	82.9		18.1	
	150	7.6	14.4	81.7		17.6	
	180	7.6	14.7	91.5		16.1	
S. Z.	Control	8.4	8.29	118		7.20	
44	PB 2.0 Gm. I.V. (21 mg./Kg.)						
	30	7.9	14.2	101		14.1	
	60	7.7	17.5	102		17.2	
	90	7.2	*21.4*	116		*18.5*	
	120	7.2	9.05	96.5		9.80	
M. K.	Control	10.5	4.68	97.7	469	4.72	0.208
45	PB 2.0 Gm. I.V. (21 mg./Kg.)						
	20	10.5	*13.2*	97.8	345	*13.5*	0.284
	35	10.3	12.0	88.6	346	13.5	0.256
	50	10.2	10.4	91.8	375	11.3	0.245
	65	10.1	8.90	108.0	372	8.25	0.290
	80	10.0	8.72	93.0	383	9.37	0.243
	110	9.8	6.22	84.9	335	7.33	0.254
	140	10.2	4.53	87.4	*328*	5.18	0.266

* Italicized values indicate peak responses to drug administration.

TABLE I—*Continued*

Subject (Age)	Period	P_{urate}	Renal clearances			Clearance ratios	
			C_{urate}	C_{In}	C_{PAH}	C_{urate}/C_{In} ×100	C_{In}/C_{PAH}
	min.	*mg. %*	*cc./min.*	*cc./min.*	*cc./min.*		
P. S.	Control	10.8	5.04	114	437	4.40	0.261
37	PB 1.5 Gm. I.V. (17 mg./Kg.)						
	30	10.0	12.8	101	337	*12.7*	0.300
	80	9.8	11.3	111	350	10.2	0.317
	110	9.6	10.9	107	*335*	10.2	0.320
	140	9.5	*13.1*	112	354	11.7	0.316
M. L.	Control	9.2	9.02	149	702	6.10	0.212
34	PB 1.5 Gm. I.V. (16 mg./Kg.)						
	15	8.9	*28.7*	146	*505*	19.0	0.289
	45	8.8	26.7	149	574	18.0	0.260
	75	8.6	27.1	125	576	21.7	0.217
	135	8.6	10.4	123	582	8.50	0.212
C. G.	Control	9.2	2.79	85.1	316	3.30	0.270
63	PB 1.0 Gm. I.V. (14 mg./Kg.)						
	30	9.2	4.91	79.9	*226*	*6.20*	0.353
	60	9.2	*5.33*	85.4	236	6.20	0.362
	90	9.2	5.03	89.4	249	5.60	0.359
	120	9.2	4.97	85.6	237	5.80	0.362
M. D.	Control	8.1	8.48	83.2	419	10.2	0.199
58	PB 1.0 Gm. I.V. (14 mg./Kg.)						
	15	8.4	*15.3*	79.5	326	19.3	0.244
	45	8.3	11.8	78.0	335	15.1	0.233
	75	8.1	12.5	81.0	327	15.4	0.248
	105	7.9	9.77	78.2	*321*	12.5	0.244
	135	7.8	9.56	80.0	366	12.0	0.218
	165	7.6	9.94	79.1	—	12.6	—
	195	7.5	10.4	83.8	377	12.4	0.222
P. M.†	Control	7.3	6.84	124	311	5.50	0.399
73	PB 1.0 Gm. I.V. (12 mg./Kg.)						
	60	7.3	7.68	106	*232*	7.20	0.457
	90	7.2	*9.60*	114	271	*8.40*	0.421
	120	7.2	8.20	132	308	6.20	0.428
	150	7.2	5.97	96.8	235	6.20	0.412

† In addition to gout this patient had coronary heart disease with mild congestive failure.

for us by Dr. J. J. Burns of the New York University Research Division at Goldwater Memorial Hospital. This was accomplished by extracting phenylbutazone from acidified plasma into heptane. It was then returned to aqueous alkali in which optical density was determined in a UV spectrophotometer at a wave length of 265 mμ.

RESULTS

The *acute effects of intravenous phenylbutazone administration* upon renal urate and inulin clearances and plasma urate concentrations in ten subjects are presented in Table I. A significant elevation of the urate clearance, C urate, and of the ratio of urate clearance to inulin clearance times 100, C urate/C_{In}, followed the injection of phenylbutazone in every instance. C urate rose from a mean control value of 6.3 cc. per min. to a mean peak value of 19.3 cc. per min., and C urate/C_{In} rose from a mean control of 6.04 to a mean peak of 19.5. In the five subjects receiving larger doses of the drug ($>$ 20 mg. per body weight), C urate rose from a mean value of 6.17 to a mean peak value of 24.2 cc. per min., and C urate/C_{In} rose from a mean of 6.23 to 25.4. In the five subjects receiving the drug in doses less than 20 mg. per Kg. body weight, C urate rose from a mean control of 6.43 to a mean peak of 14.4 cc. per min.; the corresponding C urate/C_{In} rose from 5.90 to 16.4.

Of the five subjects receiving the drug in dosages $>$ 20 mg. per Kg body weight and showing a more marked uricosuric response, four exhibited a fall in plasma urate of 1.0 mg. per cent or more. Only one of the five subjects receiving phenylbutazone in dosages $<$ 20 mg. per Kg. body weight, with less marked uricosuria, had a decline

TABLE II

Effect of a single intravenous injection of phenylbutazone (PB) over a 20 to 22 hour period
(Patients listed in descending order of drug dosage/Kg. body weight)

Case	PB dose		$C_{urate}/C_{Cr} \times 100$				Purate				Creatinine			
	Total Gm.	mg./Kg.	Control	2 hrs. after PB*	% increase	Mean, 20–22 hrs. after PB†	% increase	Control mg. %	End of 2 hrs. mg. %	% decrease	End of 20–22 hrs. mg. %	% decrease	Control	First 2 hrs.† cc./min.	% change	Mean, 20–22 hrs.† cc./min.	% change
T. T.	2.0	35	4.69	19.6	319	12.3	162	8.6	6.2	28	6.2	28	80.6	58.2	−28	60.6	−25
L. W.	2.0	27	4.12	20.2	390	10.6	157	8.3	7.1	15	6.0	28	103	96.8	−6	114	+11
S. H.	2.0	26	5.89	21.7	269	16.0	172	8.4	7.0	17	5.8	31	111	97.1	−13	95.2	−14
J. S.	2.0	25	5.35	10.1	89	10.3	93	9.2	8.6	7	5.6	39	127	113	−11	115	−9
M. S.	1.8	24	8.35	19.1	129	11.3	35	8.6	7.5	13	7.4	14	67.2	70.5	−5	66.1	−2
L. R.	2.0	21	7.90	13.3	68	11.9	51	9.3	8.9	4	7.1	24	93.2	88.3	−5	98.4	−4
S. Z.	2.0	21	7.02	17.8	153	10.9	55	8.4	7.7	8	6.6	21	97.8	87.3	−11	115	+17
M. K.	2.0	21	4.52	9.40	108	5.9	31	10.5	10.1	4	9.5	10	111	97.1	−13	95.2	−14
P. S.	1.5	17	4.90	13.1	167	7.8	59	10.8	9.5	12	7.9	23	102	97.1	−5	107	+5
M. D.	1.0	14	9.40	15.6	6	10.6	13	8.1	7.3	10	7.2	11	89.9	86.8	−4	81.5	−9

* These values represent the means of 4–8 clearance periods.
† These values represent the means of 8 or 9 clearance periods.

in serum urate concentration of more than 1 mg. per cent during the two-hour observation period.

The *continued effects* of a single intravenous injection of phenylbutazone on C urate and C_{Cr} for a period of 20 to 22 hours are summarized in Figure 1 and Table II. It is evident that the peak elevation in C urate/C_{Cr} occurred during the first two to four hours after drug administration, ranging from + 64 per cent to + 390 per cent with a mean of + 176 per cent. A significant elevation of this ratio continued for the subsequent 20 to 22 hours, ranging from + 13 per cent to + 172 per cent and averaging + 87.8 per cent. This effect again was more marked with the higher drug dosages. In all of the subjects a significant fall in plasma urate concentration occurred by the end of 20 to 22 hours, the mean fall being from 9.0 mg. per cent to 6.9 mg. per cent (Table II). The magnitude of this response could be correlated with the mean rise in C urate/C_{Cr} during this time interval.

The *effects of orally administered phenylbutazone,* given in a daily dosage of 800 mg., upon the 24-hour urinary urate excretion and the plasma urate concentration are summarized in Table III. The mean control urinary urate excretion in the eight gouty patients studied ranged from 198 to 658 mg. per 24 hours. In four subjects, uricosuria appeared during the first day of drug administration, in two subjects on the second day, and in two subjects on the third day. The peak uricosuria occurred on the first day in one patient, on the second day in one, and on the fourth day in six. In all save one subject there was an overall increase in urinary urate excretion throughout the total period of medication, the mean increase ranging from + 23 per cent to + 168 per cent. The one subject who did not show a continued uricosuric response, Case R. R., had received phenylbutazone elsewhere for several months shortly before instituting the present study. In this subject, increased urinary urate excretion occurred on the first day of medication only, 632 mg. per 24 hours, as compared to a control value of 534 mg. In spite of absence of any further uricosuric action of phenylbutazone, a plasma urate concentration of 4.6 mg. per cent was observed on the fourth day, the control value being 8.1 mg. per cent.

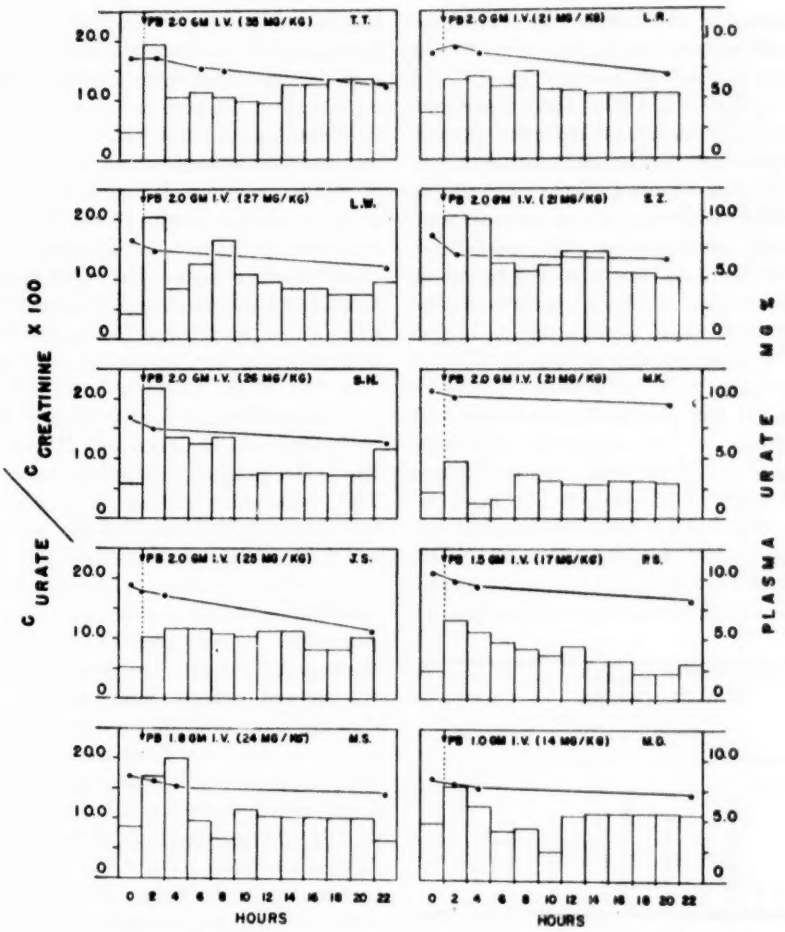
PB 2.0 GM I.V. (38 MG/KG) T.T.
PB 2.0 GM I.V. (21 MG/KG) L.R.
PB 2.0 GM I.V. (27 MG/KG) L.W.
PB 2.0 GM I.V. (21 MG/KG) S.Z.
PB 2.0 GM I.V. (26 MG/KG) S.H.
PB 2.0 GM I.V. (21 MG/KG) M.K.
PB 2.0 GM I.V. (25 MG/KG) J.S.
PB 1.5 GM I.V. (17 MG/KG) P.S.
PB 1.0 GM I.V. (24 MG/KG) M.S.
PB 1.0 GM I.V. (14 MG/KG) M.O.
C URATE / C CREATININE X 100
PLASMA URATE MG %
HOURS
HOURS

FIG. 1. THE EFFECTS OF A SINGLE INTRAVENOUS INJECTION OF PHENYLBUTAZONE (INDICATED BY ARROW) ON C URATE/$C_{CREATININE}$ (BARS) AND PLASMA URATE CONCENTRATIONS (POINTS CONNECTED BY LINE) OVER PERIOD OF 20 TO 22 HOURS IN TEN GOUTY SUBJECTS

The *relationship between phenylbutazone plasma concentrations and uricosuria* was studied in five gouty subjects. In Figure 2 (Cases L. R. and J. S.), the effect of a gradual rise in plasma phenylbutazone concentrations upon the C urate/C_{In} is illustrated. It is evident that significant elevation of the C urate/C_{In} above control values starts at plasma phenylbutazone concentrations of about 10 mg. per cent, and that stepwise elevation of the ratio occurs with rising concentrations of phenylbutazone in the plasma. The slow decline in plasma concentrations of the drug and the continued elevation of the C urate/C_{Cr} for 20 to 22 hours are apparent.

In the three subjects given rapid intravenous infusions of 2 Gm. phenylbutazone (21 to 27 mg. per Kg. body weight) the plasma phenylbutazone concentrations averaged 20 mg. per cent approximately five minutes after completion of the infusion. Significant elevations of C urate and C urate/C_{In} occurred in each instance during the first 20-minute period (Table I, Cases M. K., L. W., and S. H.).

The *glomerular filtration rate*, as measured by

inulin clearance over a two-hour period, was not affected by phenylbutazone in six subjects but was somewhat lowered in four (Table I). In subject M. L., the GFR fell to 129 cc. per min. from a control value of 149 cc. per min. approximately 75 minutes after drug administration. In subject P. M., with a control value of 124 cc. per min., the GFR was 106 cc. per min. some 60 minutes after drug administration. In cases S. Z. and S. H., the GFR declined from 118 to 101 cc. per min. and from 96.6 to 70.6 cc. per min. 30 and 125 minutes after drug administration, respectively. These represent depressions in GFR of 14 to 31 per cent, with no correlation between changes in GFR and phenylbutazone dosage. A slight depression of the endogenous creatinine clearance (10.7 to 27.8 per cent) occurred during the first two hours in five subjects (M. K., J. S., S. H., T. T., and S. Z.). This effect continued in three of these subjects for 20 to 22 hours (Table II). The most marked decline was noted in subject T. T., who received the largest dose of phenylbutazone (35 mg. per Kg. body weight), with a depression of C_{Cr} from a control of 80.6 cc. per min. to a two-hour value of 58.2 cc. per min. and a mean 20 to 22 hour value of 60.6 cc. per min.

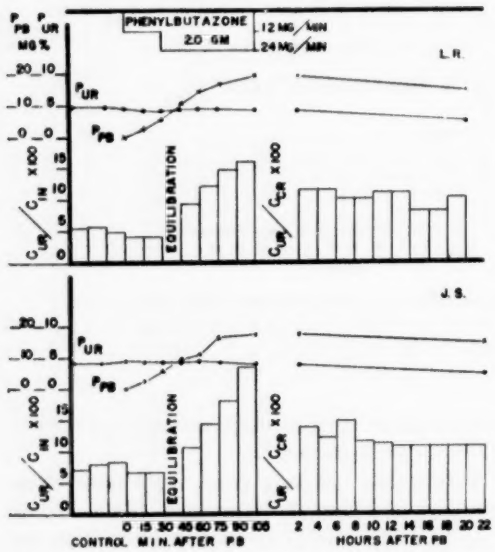

FIG. 2. THE EFFECTS OF A GRADUAL RISE IN PLASMA PHENYLBUTAZONE CONCENTRATION (CROSSES), BROUGHT ABOUT BY SLOW INTRAVENOUS INFUSION OF THE DRUG, ON C URATE/$C_{CREATININE}$ (BARS) AND PLASMA URATE CONCENTRATIONS (DOTS CONNECTED BY LINE)

A uricosuric effect was not elicited until the plasma phenylbutazone concentration reached approximately 10 mg. per cent.

Depression of the *PAH clearance* occurred in all subjects so studied, the mean control value of 495 cc. per min. falling to a mean minimum value of 326 cc. per min. (Table I). Concomitant elevations of the filtration fraction, C_{In}/C_{PAH}, occurred in all subjects. This effect upon C_{PAH} was noted at all utilized dosages of the drug from the time of the first urine collection period after drug administration until termination of the study.

In two of the subjects, S. Z. and M. S., *the effect upon* Tm_{PAH} of intravenously administered phenylbutazone in dosages of 21 and 24 mg. per Kg. body weight, respectively, was determined. The results were striking: Tm_{PAH} fell from control values of 75.7 and 61.4 mg. per min. to 12.9 and 14.0 mg. per min., respectively.

The *effects of intravenous phenylbutazone administration on the urinary excretion of sodium, potassium, chloride and inorganic phosphate in three gouty subjects* are presented in Figure 3. All showed definite inhibition of sodium and chloride excretion during drug administration which persisted for three days after discontinuance of medication. Thus, in subject O. G., the mean sodium excretion fell from a control value of 0.81 Gm. per day to 0.37 Gm. per day during medication, rising to 0.61 Gm. per day after discontinuance of the drug. The corresponding figures for chloride excretion were 1.63, 0.92, and 1.20 Gm. per day, respectively. In subject L. W. sodium excretion fell from 2.20 Gm. per day to 1.13 and 1.22 Gm. per day during and after medication. The corresponding chloride excretion was 3.61, 1.80, and 2.08 Gm. per day. In subject A. R., the values for sodium excretion were 1.25 in the control period, and 0.40 and 1.02 Gm. per day; those for chloride excretion were 2.10, 0.98, and 2.01 Gm. per day, respectively. No consistent or significant effect was observed on potassium and inorganic phosphate excretion, or on the clearance of endogenous creatinine in these three subjects.

Figure 4 summarizes the diurnal variations in the clearance ratios C_{Na}/C_{Cr}, C_K/C_{Cr}, C_{Cl}/C_{Cr}, C_P/C_{Cr}, and C urate/C_{Cr} in subject O. G. during

control, medication, and post-medication periods. The depression of C_{Na}/C_{Cr} and C_{Cl}/C_{Cr} and the elevation of C urate/C_{Cr} during the medication and post-medication periods are obvious. Significant changes in the C_K/C_{Cr} and C_P/C_{Cr} were not noted. Essentially similar results were obtained for the other two subjects. Significant changes in body weight and 24-hour urine volume were not noted during these studies.

In subject L. W., the *plasma sodium concentration* rose from a control value of 145 mEq. per L. to 156 mEq. per L. six hours after the first dose of phenylbutazone. This cannot be accounted for by the very small amount of sodium (sodium salt of phenylbutazone) infused. Return to the control value occurred by the next 24 hours. In subject O. G., the plasma sodium concentration rose from a control value of 141 mEq. per L. to 145 mEq. per L. and 150 mEq. per L., six and twelve hours after start of medication. Normal values were regained 18 hours later. This phenomenon was not observed in subject A. R.

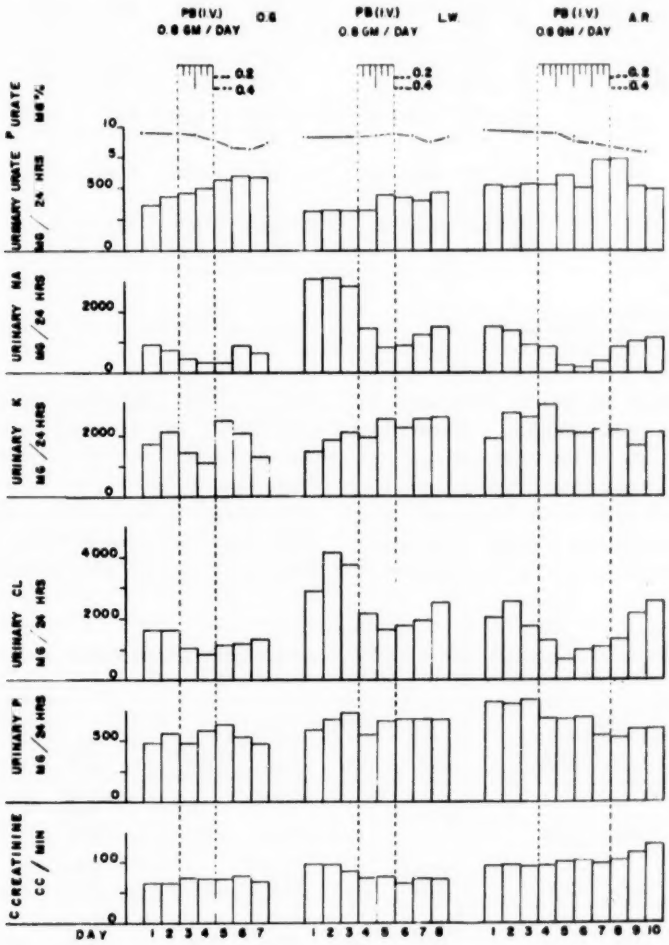

FIG. 3. THE EFFECTS OF INTRAVENOUS INJECTIONS OF PHENYLBUTAZONE UPON THE URINARY EXCRETION OF SODIUM, POTASSIUM, CHLORIDE, INORGANIC PHOSPHATE AND URIC ACID, AND UPON THE ENDOGENOUS CREATININE CLEARANCE IN THREE GOUTY SUBJECTS

TABLE III

Effect of orally administered phenylbutazone (800 mg./day) on urinary urate excretion and plasma urate concentration in eight gouty subjects

Subject (Age)	Tophi	C_{Inulin}		Control period Mean	Control period Range	Days on phenylbutazone 1	2	3	4	5	6	7
		cc./min.										
T. T.	+++	76.8	U	198	184–227	313*	473	413	938†	680		
61			P	9.1		8.4	8.0	7.9	7.2	6.7		
L. W.	0	92.8	U	306	243–328	316	337	542*	812†	796	704	
44			P	6.6					4.7		5.3	
O. G.	0	74.1	U	457	393–498	628*	756	777	796†			
46			P	7.3			5.4		4.4			
H. S.	+	89.0	U	658	618–699	843*	1028	880	1170†			
49			P	8.9					4.7			
L. G.	0	111	U	640	547–742	532	1289†	869	840	1080	1083	740
54			P	7.4		7.0	7.3	7.8	5.6	4.4		3.9
M. H.	0	74.5	U	489	466–513	505	653*	632	911†			
42			P	8.7		8.1	7.3	6.3	4.1			
G. B.	++++	48.9	U	457	430–511	402	394	554*	905†			
64			P	9.8					7.5			
R. R.	0	—	U	534	480–582	632†	417	242	595	587	470	504
44			P	8.1					4.6			5.3

* Initial uricosuria.
† Maximum uricosuria noted.
U = urinary urate, mg./24 hours.
P = plasma urate, mg. %.

Significant changes in plasma potassium, inorganic phosphate and chloride concentrations were not noted in any of the three subjects.

The effect of *simultaneous administration of phenylbutazone and probenecid* (*Benemid*) upon urinary urate excretion was studied in seven subjects. The administration of phenylbutazone orally, 0.2 Gm. three times a day, to three subjects during effective probenecid uricosuria (1.0 Gm. probenecid per day) resulted in a moderate depression of the 24-hour urate excretion for one or two days. This was followed by return to previous levels of urate excretion in spite of continued simultaneous administration of both drugs.

TABLE IV

*The effect of simultaneous administration of benemid (BEN) and phenylbutazone (PB) upon the renal clearances of urate and inulin**

Subject	Control period C_{In}	Control period C_{urate}	Control period $C_{urate}/C_{In}\times100$	Post-medication period I Drug given	Post-medication period I C_{In}	Post-medication period I C_{urate}	Post-medication period I $C_{urate}/C_{In}\times100$	Post-medication period II Drug given	Post-medication period II C_{In}	Post-medication period II C_{urate}	Post-medication period II $C_{urate}/C_{In}\times100$
	cc./min.	*cc./min.*		*mg./Kg.*	*cc./min.*	*cc./min.*		*mg./Kg.*	*cc./min.*	*cc./min.*	
P. C.	89.3	5.54	6.19	(BEN) 20.0	83.7	19.2	22.9	(PB) 14.0	77.0	13.2	17.2
I. G.	109	8.05	7.38	(BEN) 20.0	111	25.4	22.9	(PB) 13.3	98.1	20.1	20.5
C. G.	85.1	2.79	3.28	(PB) 14.0	85.1	5.06	5.95	(BEN) 20.0	85.0	10.4	12.3
P. M.	110	6.85	6.23	(PB) 12.0	95.0	7.86	8.28	(BEN) 20.0	91.0	21.4	23.6

* All clearance values represent the mean of at least three clearance periods.

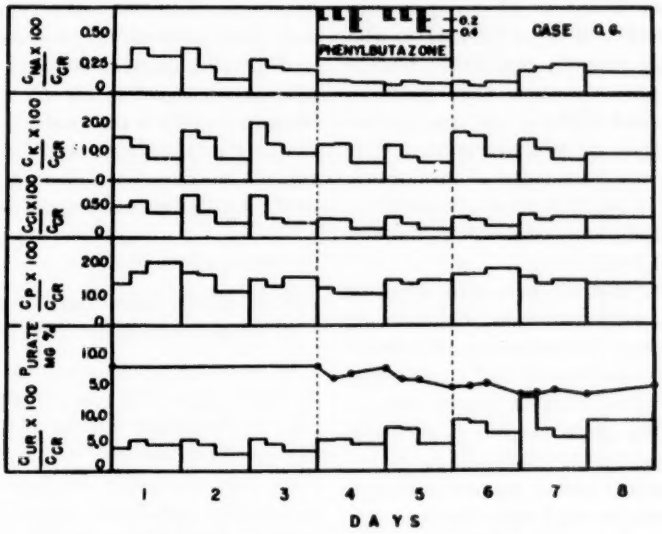

FIG. 4. THE EFFECTS OF INTRAVENOUS INJECTIONS OF PHENYLBUTAZONE UPON THE DIURNAL VARIATIONS IN CLEARANCE RATIOS OF C_{Na}/C_{CR}, C_K/C_{CR}, C_{Cl}/C_{CR}, C_P/C_{CR}, AND C URATE/C_{CR}

In four subjects the acute effects of concomitant intravenous administration of both drugs upon inulin and urate clearances were determined by giving 1 Gm. of probenecid prior to phenylbutazone in two of the subjects, and subsequent to phenylbutazone in the remaining two. The results are presented in Table IV. Subjects P. C. and I. G. received intravenous probenecid first. Their C urate/C_{In} ratios rose from 6.19 and 7.38 to 22.9 and 22.9, respectively, without any changes in C_{In}. When phenylbutazone was then administered, slight depressions in C_{In} occurred. The slight declines noted in C urate/C_{In} may be explained on the basis of the time required for the experimental procedure, the peak uricosuric effect of probenecid having subsided. In subjects C. G. and P. M., the prior administration of phenylbutazone resulted in a slight decline of C_{In} in P. M. only, and a slight elevation of C urate/C_{In} in both. Subsequent probenecid administration resulted in additional elevations of these ratios without further changes in C_{In}. Marked interference with probenecid uricosuria by phenylbutazone administration is not apparent from these studies.

Untoward effects attributable to phenylbutazone administration were not observed in any of the subjects included in this study.

DISCUSSION

Uricosuric effect

Phenylbutazone in adequate dosage increased the urate clearance and enhanced urinary urate excretion in most gouty subjects studied. The uricosuric effect, in general, appeared to be related to the plasma phenylbutazone concentration. In the patients studied a plasma phenylbutazone concentration of approximately 10 mg. per cent was required to induce uricosuria and the uricosuric effects increased roughly in proportion to further elevation of drug levels in the blood. Immediate uricosuric plasma concentrations are readily achieved by intravenous injection of adequate doses. Thus, in three subjects, given 2.0 Gm. of phenylbutazone intravenously, the initial plasma drug levels five minutes after injection averaged 20 mg. per cent and were still as high as 14 mg. per cent 20 hours later. In most subjects receiving the usual daily dose, 0.8 Gm. by mouth, the plasma concentration of the drug, while variable from individual to individual, (6)

usually ranges from 6 mg. to 15 mg. per cent. For example, in subject M. H. (Table III), who received this dosage daily by mouth, the plasma concentrations of phenylbutazone were found to be 6.9, 10.6, 14.4, and 15.2 mg. per cent on the four successive days of administration. Uricosuria started on the second day of medication, when the plasma phenylbutazone concentration was 10.6 mg. per cent, and became more marked as the plasma drug levels rose.

The data indicate that the uricosuric effect of phenylbutazone is attributable to inhibition of tubular reabsorption of filtered urate. C urate/C_{In} was found to be consistently and significantly elevated by the drug in adequate plasma concentration. The plasma urate in gout is virtually completely filtrable at the glomerulus, so far as we have been able to determine by ultrafiltration studies to be reported in detail elsewhere.

The observations of Burns and his associates (6, 7, 8) on the disposition of phenylbutazone in man appear to shed some light on the relationship between plasma phenylbutazone concentration and inhibition of tubular reabsorption of urate. These investigators found that, at plasma phenylbutazone concentrations less than 10 mg. per cent, the drug is almost completely bound to plasma proteins; at levels of 10 mg. per cent only 2 per cent of the drug is filtrable and, as the plasma concentration rises, more and more of the drug is filtrable. Since very little unmetabolized phenylbutazone is found in the urine (7), the freely filtrable fraction of the drug must be practically completely reabsorbed by the renal tubules. This reabsorptive process may interfere in some way with the transfer mechanisms for urate reabsorption.

The inhibition of tubular reabsorption of urate by phenylbutazone appears to be significantly less than that of comparable doses of probenecid. Thus in acute experiments with probenecid (4), doses of 20 mg. per Kg. body weight produced a mean rise in C urate from a control of 8.55 cc. per min. to 32.9 cc. per min., and a rise in C urate/C_{In} from a mean control value of 7.10 to 33.7. Under the same experimental conditions, phenylbutazone in doses greater than 20 mg. per Kg. body weight produced much lower peak responses (Table I).

Because of the more pronounced and regular uricosuric effect of probenecid, and its insignificant predisposition to serious toxic reactions, probenecid is more suitable for protracted use as a uricosuric agent in chronic gout than phenylbutazone, which is not recommended for the purpose. Phenylbutazone may be employed to advantage in short-term therapy to control acute gouty seizures in many cases, however, since this action of the drug does not depend upon its uricosuric effect (1, 2).

Fall in plasma urate concentration and its relationship to uricosuria

In accordance with the general experience, we find that phenylbutazone in adequate dosage effects a significant fall in plasma urate levels. The question has been raised whether such declines can reasonably be accounted for by the concomitant increase, if any, in urinary urate excretion. Kidd, Boyce, and Freyberg (9), for example, noted a distinct decrease in plasma urate in 15 gouty subjects receiving 400 to 800 mg. phenylbutazone per day, but in nine of these patients, apparently on uncontrolled diets, they were unable to demonstrate a significant increase in urinary urate excretion.

Any attempt to relate changes in plasma urate concentration to the quantity of urate excreted in the urine is, for the present, subject to large error since this relationship is markedly affected by such variables as the size of the readily mobilizable tissue deposits (miscible pool) of urate in individual gouty subjects, fluctuations in urate biosynthesis due to intrinsic and extrinsic (dietary) causes, alterations in plasma volume, variations in renal clearance of urate, etc. If the readily mobilizable tissue deposit of urate is very small, or has been markedly depleted by prior treatment with a uricosuric agent, exhibition of any uricosuric drug will elicit only a small and transitory increase in urinary urate excretion and this will be accompanied by a distinct and sustained fall in plasma urate levels, reflecting the failure of replenishment from mobilizable tissue (tophaceous) reserves. This has been our invariable experience with probenecid and the observations on Case R. R. in the present study (Table III) would suggest that the same applies to phenylbutazone.

In our patients receiving phenylbutazone intravenously, the fall in plasma urate concentra-

tion and the extent of uricosuria were consistently greater in those given larger doses in terms of mg. per Kg. body weight. The correlation between uricosuric effect and fall in plasma urate levels in these subjects was reasonably good (Tables I and II, Figure 1). In the patients given phenylbutazone orally, the uricosuric response in most instances also seemed adequate to account for the fall in plasma urate levels but several exceptions were noted. Case R. R. (Table III) has already been commented upon. In Cases A. R. (Figure 3) and M. H. (Table III) there was a distinct fall in plasma urate concentration before the urinary urate excretion was appreciably increased. A factor which played a role in lowering the plasma urate concentration in Case M. H. was the expansion of extracellular fluid volume consequent upon retention of salt and water. In this subject measurement of T-1824 space revealed a 47 per cent increase 96 hours after medication was begun, although the 48-hour measurement showed no increase. Chenkin *et al.* (10) have demonstrated expansions in plasma volume as great as 50 per cent in some patients receiving the drug.

Other possible explanations for a fall in plasma urate concentration apparently disproportionate to the uricosuric effect of phenylbutazone include depression of urate biosynthesis, accelerated removal of urate from the blood by excretory pathways other than renal, deposition in the tissues or a shift of urate into the intracellular fluid. There is no evidence, as yet, for such effects. We have excluded the possibility of interference of the drug with the development of color in our method of urate analysis (see under Methods).

Effect on glomerular filtration rate, electrolyte excretion and renal blood flow

In eight of our thirteen patients given 1 to 2 Gm. phenylbutazone intravenously there was no reduction in glomerular filtration rate as measured by inulin or endogenous creatinine clearance; the remaining five patients showed slight depression, most marked in the subject receiving the largest dose on the basis of body weight (35 mg. per Kg.). This is in agreement with the findings of Wilkinson and Brown (11), who noted no change in endogenous creatinine clearance, and Dupont, Duckert-Maulbetsch, and Fabre (12), who reported a mean depression in 24-hour endogenous creatinine clearance from 109 to 99 cc. per min. in eight subjects receiving intramuscular injections of 1 Gm. of phenylbutazone daily.

The retention of sodium and chloride uniformly noted in our experiments is in accord with the observations of previous investigators (10–14). That the decrease in excretion of these ions is primarily the result of increased tubular reabsorption is indicated by the significant reduction in C_{Na}/C_{In} and C_{Cl}/C_{In}. A similar conclusion was reached by Dupont, Duckert-Maulbetsch, and Fabre (12) in their study on non-gouty subjects. The absence of any significant effect on potassium excretion agrees with the experience of others (10–14).

The rapid, marked and sustained depressions in PAH clearance noted by us are attributable to decreased renal tubular extraction of PAH rather than to reduction in renal blood flow, as indicated by marked decreases in Tm_{PAH}. Similar findings have been recorded by Berthoud (15) in connection with intravenously administered Irgapyrine. It is interesting to note that the effect of phenylbutazone in this respect is as marked as that of probenecid, whereas the associated uricosuric effects are much less pronounced. This dissociation would suggest that the mechanism of action of the two drugs on renal transport systems differs.

SUMMARY AND CONCLUSIONS

1. In ten gouty subjects receiving phenylbutazone intravenously in dosages ranging from 12 to 27 mg. per Kg. body weight, C urate rose from a mean control value of 6.30 cc. per min. to a mean peak value of 19.3 cc. per min., and C urate/C_{In} × 100 rose from a mean control of 6.04 to 19.5 within two hours. Elevation of C urate/C_{Cr} continued for 20 to 22 hours. Uricosuria occurred in all ten subjects and the magnitude of this effect appeared to correlate with dosage.

2. In two patients receiving a slow intravenous infusion of phenylbutazone, elevations of C urate/C_{In} were first noted at plasma phenylbutazone concentrations of approximately 10 mg. per cent. Stepwise elevation of C urate/C_{In} occurred with rising drug concentrations in the plasma.

3. Eight gouty subjects were given phenylbutazone orally in dosages of 800 mg. per day. Seven showed increased urinary urate excretion

but the duration and magnitude of this response was variable. All of the subjects exhibited a decrease in plasma urate concentration. In three an apparent inconsistency was observed between the degree of uricosuria and the decline in plasma urate concentration. Possible explanations are presented.

4. The glomerular filtration rate was unaffected in eight of thirteen gouty subjects following intravenous administration of phenylbutazone, the remainder exhibiting a slight depression in GFR. This effect continued for 20 to 22 hours in three of the subjects. The most profound depression in GFR, from 80.6 to 60.6 cc. per min. occurred in the subject receiving the largest dose, 35 mg. per Kg. body weight.

5. A depression in C_{PAH} from a mean control value of 495 cc. per min. to a mean minimum value of 326 cc. per min. occurred in ten subjects receiving phenylbutazone intravenously. This effect appeared rapidly and was sustained throughout the period of observation. In two subjects so studied the Tm_{PAH} fell from control values of 75.4 and 61.4 mg. per min. to 12.9 and 14.0 mg. per min., respectively.

6. In three subjects intravenous phenylbutazone in doses of 800 mg. per day produced depressions of C_{Na}/C_{Cr} and C_{Cl}/C_{Cr} without appreciable changes in C_K/C_{Cr} and C_P/C_{Cr}. There was significant sodium and chloride retention.

7. The simultaneous administration of phenylbutazone and Benemid did not result in marked inhibition of the uricosuric effect of either drug.

8. The data indicate that the principal renal effects of phenylbutazone are upon tubular transport mechanisms, inhibiting the reabsorption of urate and the secretion of PAH, and increasing the reabsorption of sodium and chloride.

9. Despite its uricosuric action, phenylbutazone is not recommended for protracted use as a uricosuric agent in chronic gout since its enhancement of urinary urate excretion is distinctly less than that of equivalent doses of probenecid and it is much more prone to produce serious toxic reactions.

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HEXAMETHONIUM IN THE CHRONIC TREATMENT OF HYPERTENSION—ITS EFFECT ON RENAL HEMODYNAMICS AND ON THE EXCRETION OF WATER AND ELECTROLYTES [1]

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Neurogenic (autonomic) factors in hypertensive vascular disease (1, 2) may be studied with hexamethonium [2] which reduces arterial blood pressure by blockade of the sympathetic ganglia. When the blood pressure is reduced acutely in this manner, alterations in renal hemodynamics have been observed. Following a single dose of hexamethonium administered parenterally to patients with hypertension, the glomerular filtration rate and renal plasma flow are immediately depressed but within one hour renal hemodynamic readjustment occurs and both functions return to or towards control values, although the arterial blood pressure remains depressed (3, 4) for two to six hours. The present study was designed to evaluate the effects of prolonged reduction of blood pressure in hypertensive patients, with and without renal damage, during the therapeutic oral administration of hexamethonium. In addition to studies in the supine position, observations on renal hemodynamics were made during ambulation before and during therapy. The latter observations were made in order to evaluate the effect of ganglionic blockade on the renal vasoconstriction which normally is associated with changing from the supine to the upright position (either static tilt or ambulation) in the untreated patient (5).

METHODS

Seven patients with hypertension were studied by measurements of glomerular filtration rate (inulin clearance), renal plasma flow (para-aminohippurate clearance), and maximum tubular excretory capacity (TmPAH). The rates of urine formation, sodium excretion, potassium excretion, and the changes in blood pressure were observed. The techniques of renal function and the methods of analysis have been previously described (3). The control determinations of glomerular filtration rate (GFR) and renal plasma flow (RPF) consisted of three consecutive 10-minute periods in recumbency, followed by three periods in the ambulatory state. During the ambulatory studies the patients pushed the equipment mounted on a small table equipped with wheels. Following these six periods and after a 60-minute equilibration and rest period in the recumbent position, three control determinations of GFR and TmPAH were performed. These were followed by three periods in the ambulatory state. The patients were then given hexamethonium orally four times daily to establish a reduction in mean blood pressure of 40 mm. Hg or more when in the upright position. The second group of studies ("drug" studies) were done after one month or more and after at least seven days of consistent reduction of blood pressure. The renal function studies were then repeated in precisely the same fashion as the control studies, recumbent and ambulatory. Supine studies on GFR and RPF were done on an additional six patients (XM to XCa, Tables I and II) who were treated in a similar fashion. Determination of TmPAH was done on one patient in this latter group. Blood pressures were determined on the first seven patients with a sphygmomanometer and on the last six patients by intra-arterial manometry.

Observations on the effect of ambulation on electrolyte excretion were made on one additional patient (Table IV) who received hexamethonium as oral therapy for hypertension and who had an adequate response in blood pressure reduction in the upright position. He was studied in the metabolic ward and was given a constant diet in terms of calories (2,300), protein (70 Gm.), sodium (800 mg. NaCl), potassium (700 mg.), chloride (800 mg. NaCl), and water (3,000 ml.). After a five-day equilibration period, the urine was collected in four-hour periods for three consecutive days. It was analyzed for chloride, sodium, and potassium. The patient was ambulatory for three periods and recumbent for three periods during each day.

RESULTS

The patients required an average oral dose of 875 mg. of hexamethonium every six hours in or-

[1] Supported in part by a grant from the National Institutes of Health, United States Public Health Service.

[2] Supplied through the courtesy of Burroughs Wellcome and Company and Ciba Pharmaceutical Products, Inc.

der to obtain a consistent lowering of blood pressure. The percentile reduction in systolic pressure was equal to the percentile reduction in diastolic pressure. A typical study is summarized in Figure 1.

I. Effect of oral hexamethonium on renal hemodynamics in the supine position

Hexamethonium produced an average reduction of mean blood pressure (MBP) to 78 per cent of the control (from 162 to 127 mm. Hg) observations. This was not associated with a significant change in glomerular filtration rate (GFR) (98 per cent of control), maximum tubular excretory capacity for PAH (TmPAH) (95 per cent of control), or in renal plasma flow (RPF) (116 per cent of control). The constancy of renal blood flow (RPF/1 minus hematocrit) (RBF) associated with a reduction in mean blood pressure is reflected in an average decrease in renal vascular resistance (RVR = MBP/RBF) of 28 per cent (Table II).

II. Effect of oral hexamethonium on renal hemodynamics during ambulation

During the control studies (prior to hexamethonium administration) ambulation did not alter the mean blood pressure. However, there was evidence of renal vasoconstriction as reflected in a depression of GFR, RBF, and TmPAH and an increase in RVR. Ambulation, after hexamethonium administration, caused an additional reduction of MBP from 78 per cent to 65 per cent (127 to 104 mm. Hg) of the control (162 mm. Hg) supine value (Table I). This was associated with

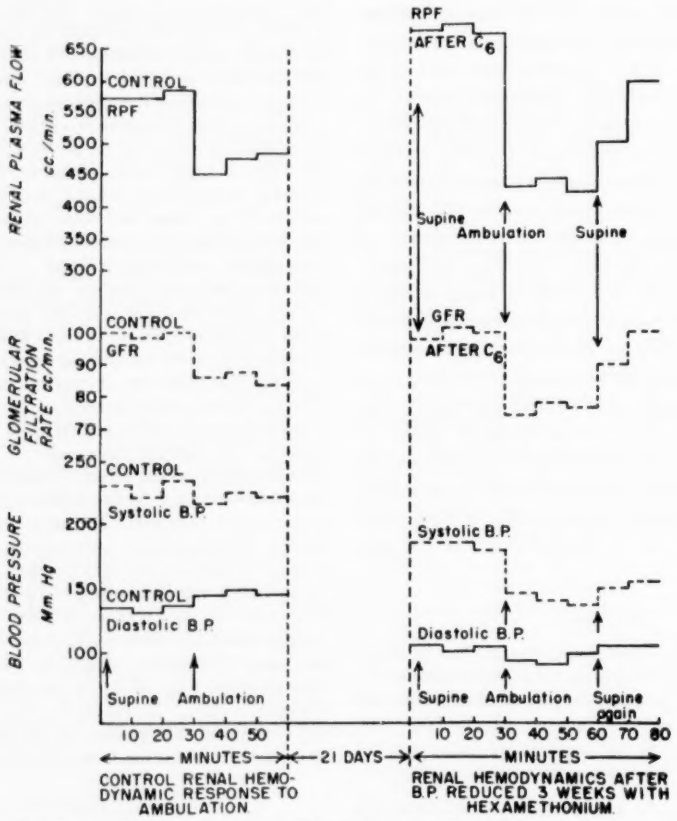

FIG. 1. A TYPICAL (PATIENT WA, TABLE I) RENAL HEMODYNAMIC AND BLOOD PRESSURE RESPONSE TO AMBULATION BEFORE AND AFTER GANGLIONIC BLOCKADE WITH HEXAMETHONIUM

TABLE I

Effect of oral hexamethonium on renal function

Pt.	Diagnosis*	Age	Mean blood pressure†				Glomerular filtration rate				Renal plasma flow				Tubular excretory capacity			
			C_R	C_A	D_R	D_A	C_R	C_A	D_R	D_A	C_R	C_A	D_R	D_A	C_R	C_A	D_R	D_A
			mm. Hg				*ml. per min.*				*ml. per min.*				*mg. per min.*			
WA	HVD	60	156	156	120	100	100	86	99	72	572	450	697	414	66	56	69	60
AR	MHVD	62	210	220	180	130	52	45	48	25	221	179	185	112	35	23	40	28
TE	HCVD	56	160	160	130	90	90	86	83	61	445	387	477	234	72	71	76	77
JR	HVD	58	123	133	105	85	67	73	70	72	570	509	752	367	62	57	58	53
CA	HCVD	67	147	147	120	90	78	69	81	77	728	680	631	451	83	85	81	74
JM	HCVD	60	160	150	140	110	52	33	49	42	255	177	220	174	48	47	41	33
AB	HVD	42	160	160	140	120	74	63	64	46	355	280	383	250	77	62	57	48
XM	MHCVD	54	184		130		78		79		412		477		52		43	
XH	MHCVD	36	150		120		74		84		332		412					
XC	MHCVD	42	164		123		69		72		300		340					
XB	HVD	44	178		110		93		86		520		610					
XJ	HVD	54	145		105		132		129		536		711					
XCa	MHCVD	34	175		126		67		65		175		313					
Mean			162	161	127	104	79	65	78	56	417	380	478	286	62	57	58	53
Mean % of supine control‡				101	78	65		88	98	77		82	116	63		89	95	83

* HVD—hypertensive vascular disease
MHVD—malignant hypertensive vascular disease
HCVD—hypertensive cardiovascular disease
MHCVD—malignant hypertensive cardiovascular disease

† Mean blood pressure = diastolic pressure plus one-third of the pulse pressure.
C_R—control recumbent
C_A—control ambulatory
D_R—drug recumbent
D_A—drug ambulatory

‡ Mean value for per cent of control of individual patients—control = 100%.

renal vasoconstriction and a reduction in renal functions (GFR, RPF, and TmPAH) which was more marked than with ambulation before the administration of hexamethonium. For example, ambulation caused an average decrease of 12 per cent in GFR during the control studies as compared to 23 per cent (from supine control) after hexamethonium. The RPF, which was not depressed by blood pressure reduction in the supine position, was reduced by ambulation (after hexamethonium) to 63 per cent of the supine control which was an additional decrease of 19 per cent from the ambulatory control. Ambulation caused an average reduction in TmPAH of 11 per cent during the control period as compared to 17 per cent (from 62 to 53 mg. per min.) after hexamethonium. Further evidence of renal vasoconstriction during ambulation after hexamethonium administration is found in the observation that there was a decrease in RBF of 37 per cent (725 to 501 ml. per min.) from the control supine value as compared to 15 per cent before hexamethonium. When the RBF in the supine position after hexamethonium is used as the reference point, the response to ambulation represents an even greater reduction (Table II). Ambulation after hexamethonium caused an average increase in RVR to only 105 per cent of the control, supine value. However, the percentage increase following ambulation is considerably greater when the post-drug supine value is used as the reference point.

III. Effect of oral hexamethonium on water and electrolyte excretion

Prior to hexamethonium administration, ambulation caused a reduction in urine volume and sodium excretion to 79 per cent and 75 per cent of the control supine observations, respectively, without a reduction in blood pressure. After ganglionic blockade and blood pressure reduction with hexamethonium, ambulation produced a further decrease in blood pressure by comparison to the supine post-drug value. This was associated with a sharp reduction in urine volume and in sodium excretion. The changes brought about by ambulation were of a somewhat greater magnitude (percentagewise) than those observed following ambu-

TABLE II

Effect of oral hexamethonium on renal vascular resistance

Pt.	Hematocrit*				Renal blood flow				Renal vascular resistance ×10²†			
	C_R	C_A	D_R	D_A	C_R	C_A	D_R	D_A	C_R	C_A	D_R	D_A
					ml./min. = $\frac{RPF}{1-Hct.}$							
WA	45	46	38	40	1,040	833	1,124	690	150	187	107	145
AR	46	47	46	48	409	338	343	215	513	651	525	605
TE	45	47	43	45	809	730	837	425	198	219	155	212
JR	42	43	42	43	983	893	1,297	644	125	149	81	132
CA	45	46	41	42	1,324	1,259	1,069	778	111	117	112	116
JM	42	46	43	46	440	328	386	322	364	457	363	342
AB	41	42	41	42	602	483	649	431	266	331	216	278
XM	38		40		665		795		277		164	
XH	44		43		593		723		253		166	
XC	46		46		556		630		295		195	
XB	40		41		867		1,034		205		106	
XJ	36		37		838		1,129		173		93	
XCa	41		40		297		522		589		241	
Mean	42	45	42	44	725	695	811	501	271	302	194	261
Mean % of control		103	98	100		85	115	63		120	72	105

* See Table I for definition of symbols.
† Renal vascular resistance = mean blood pressure/renal blood flow.

lation during the control period. There was no change in potassium excretion due to ambulation (106 per cent of supine control) during the control period but after hexamethonium there was an apparent decrease to 79 per cent of the supine value during hexamethonium therapy.

The patients were given a constant sodium diet for two weeks prior to receiving hexamethonium and throughout the period of drug administration; yet, after one month of therapy, all but one of the patients showed a reduction in plasma sodium concentration. The lone exception was the only patient who did not show a decrease in the excretion of sodium in the post-drug supine state as compared to the control supine value. Despite the apparent decrease in sodium and water excretion during the studies which were performed after receiving hexamethonium for one month, none of the pa-

TABLE III

Effect of oral hexamethonium on water and electrolyte excretion

Pt.	Urine volume*				Sodium excretion				Potassium excretion				Plasma concentration: Sodium		Plasma concentration: Potassium	
	C_R	C_A	D_R	D_A	C_R	C_A	D_R	D_A	C_R	C_A	D_R	D_A	C_R	D_R	C_R	D_R
	ml./min.				*γEq./min.*				*γEq./min.*				*mEq./L.*			
WA	9.4	7.4	4.3	3.9	287	191	208	87	121	138	90	77	139	134	3.3	3.2
AR	17.0	16.0	7.9	3.0	361	204	135	91	236	200	221	162	129	124	3.2	3.0
TE	27.0	11.0	8.9	3.5	565	204	104	17	146	282	95	62	140	133	3.7	3.7
JR	16.0	17.0	5.9	2.3	191	217	261	74	162	164	79	51	140	140	3.0	3.0
CA	4.5	5.6	6.8	4.2	226	200	135	39	231	269	31	28	140	132	4.1	4.2
JM	9.0	2.7	6.0	2.2	1,043	1,043	522	522	205	115	67	51	136	125	3.7	3.6
AB	10.0	7.6	8.0	3.4	870	522	130	35	128	103	26	26	140	132	2.9	3.1
Mean	13.3	9.6	6.8	3.2	506	369	214	124	176	182	87	65	138	131	3.4	3.4
Mean % of control†		79	66	50†		75	56	44†		106	50	79†		95		100

* See Table I for definition of symbols.
† Mean % of control—
$C_A = C_A/C_R \times 100$
$D_R = D_R/C_R \times 100$
$D_A = D_A/D_R \times 100$

TABLE IV

*Comparative effect of ambulation and recumbency on water and electrolyte excretion in one patient under hexamethonium (oral) therapy**

Day	Period	State	U.V.	Cl	Na	K
12–7–52	1 (7–11 a.m.)	A	1.04	55	47	22
	2 (11–3 p.m.)	R	5.17	83	333	225
	3 (3–7 p.m.)	A	3.17	58	71	20
	4 (7–11 p.m.)	A	1.96	38	42	18
	5 (11–3 a.m.)	R	4.29	92	96	75
	6 (3–7 a.m.)	R	2.33	98	63	23
12–8–52	1 (7–11 a.m.)	A	0.79	29	34	10
	2 (11–3 p.m.)	R	4.21	108	138	208
	3 (3–7 p.m.)	A	2.46	79	58	221
	4 (7–11 p.m.)	A	2.50	55	63	38
	5 (11–3 a.m.)	R	3.46	88	75	58
	6 (3–7 a.m.)	R	1.96	79	63	19
12–9–52	1 (7–11 a.m.)	A	1.08	80	58	29
	2 (11–3 p.m.)	R	5.54	107	304	254
	3 (3–7 p.m.)	A	1.38	37	29	8
	4 (7–11 p.m.)	A	1.63	48	42	14
	5 (11–3 a.m.)	R	4.42	100	154	92
	6 (3–7 a.m.)	R	1.67	89	42	12

* State: A—ambulatory; R—recumbent
U.V.: Urine volume (ml./min.)
Cl: Chloride excretion (γ Eq./min.)
Na: Sodium excretion (γ Eq./min.)
K: Potassium excretion (γ Eq./min.)

tients gained weight nor did they demonstrate signs of edema to indicate salt and water retention.

The results of the electrolyte excretion studies during alternating four-hour recumbent and ambulatory periods in one patient are indicated in Table IV. Observations were made on the excretion rates of water, sodium, chloride, and potassium. These excretion rates were depressed in the four-hour periods of ambulation but were augmented in the four-hour periods of recumbency which immediately followed the period of ambulation. The excretion rates of water and electrolytes decreased during the second four-hour period when two collection periods were run consecutively in the recumbent position.

DISCUSSION

Since hexamethonium may be used orally in long term therapy of hypertension, it is of interest to observe the changes in homeostatic balances which occur. Previous work has demonstrated that a single intravenous dose of hexamethonium is followed by a prolonged reduction in arterial pressure but only a temporary depression of glomerular filtration rate (GFR) and renal plasma flow (RPF) in the recumbent position. The present data indicate a continuation of this response during prolonged oral hexamethonium therapy. There was no depression of GFR, RPF, or TmPAH in the supine position, although the blood pressure was significantly reduced. This was reflected in a reduction of renal vascular resistance. Preexistent renal damage did not appear to alter this response. These observations are in agreement with the observations of Corcoran, Taylor, and Page (6) who observed an increase in RPF and a decrease in MBP following spinal anesthesia and suggest that in patients with hypertension a significant degree of renal vasoconstriction may be mediated over the sympathetic nervous system.

TABLE V

Summary of the effect of ambulation before and after oral hexamethonium in seven patients (Mean values and percentile changes)†*

	C_R	C_A	C_A/C_R $\times 10^2$	D_R	D_R/C_R $\times 10^2$	D_A	D_A/C_R $\times 10^2$	D_A/D_R $\times 10^2$
Mean blood pressure (mm. Hg)	159	161	101	134	84	104	65	78
Glomerular filtration rate (ml./min.)	73	65	89	71	97	56	77	79
Renal plasma flow (ml./min.)	449	380	85	478	106	286	64	60
Tubular excretory capacity (mg./min.)	63	57	90	60	95	53	84	88
Hematocrit	44	45	102	42	95	44	100	105
Renal blood flow (ml./min.)	801	695	87	815	102	501	63	61
Renal vascular resistance $\times 10^2$	247	302	122	223	90	261	106	117
Urine volume (ml./min.)	13.3	9.6	72	6.8	51	3.2	—	47
Sodium excretion (γ Eq./min.)	506	369	73	214	42	124	—	58
Potassium excretion (γ Eq./min.)	176	182	103	87	49	65	37	75

* All values are mean values for seven patients (WA to AB) who were studied both in the supine position and during ambulation.

† C_R—Control recumbent
C_A—Control ambulatory
D_R—Recumbent after hexamethonium
D_A—Ambulatory after hexamethonium

The participation of neurogenic reflexes in the control of renal functions is suggested by the changes accompanying tilting (5) or ambulation as summarized in Table V (C_A/C_R). Renal vasoconstriction occurs as evidenced by the increase in RVR and a decrease in GFR, RBF, and TmPAH, but minimal, if any, change in blood pressure. This is associated with a decrease in the excretion of water and sodium. During hexamethonium therapy, there is an additional reduction in blood pressure due to ambulation which is also accompanied by a reduction in GFR, RBF, TmPAH, and the excretion of water, sodium, and potassium (Table V, D_A/D_R). There is very little increase in RVR in the ambulatory state after hexamethonium as compared to the recumbent state before the drug is administered (D_A/D_R). However, when the recumbent state after ganglionic blockade is used as the baseline there is nearly as great a percentile increase in RVR due to ambulation as there was in the untreated patient (Table V, D_A/D_R as compared to C_A/C_R, respectively). This indicates that under the stress of postural changes, reflex vasoconstrictive mechanisms in the kidney are retained despite partial ganglionic blockade with hexamethonium. In spite of the (apparent) depression of renal hemodynamics during ambulation there was no instance of increased renal decompensation as manifested by a rising blood urea nitrogen.

The explanation for the depression of water and electrolyte excretion during hexamethonium therapy is not apparent. Even during recumbency, the present data show a decrease in water and sodium excretion during hexamethonium therapy as compared to the control observations. The reduction in plasma sodium concentration suggests a partial depletion of this electrolyte during the administration of hexamethonium. A depleted state due to sodium loss during the initial administration of hexamethonium might be reflected in decreased excretion of sodium in the urine after receiving hexamethonium for prolonged periods (one month or more). The initial response to hexamethonium administration was not studied since this observation on sodium excretion was not anticipated. Variations in sodium and water intake between the control study and one month later may also be responsible for the reduced sodium excretion since sodium intake was not rigidly controlled in these patients. The amount of sodium in the diet was kept constant during the study and for a two-week control period before the drug was administered. However, one cannot be sure that the patients consumed their entire diet. This may well be a factor since initial therapy with hexamethonium is frequently associated with a decreased appetite. Before any conclusions are drawn in this regard the problem should be investigated in more detail and the observations confirmed by other investigators.

The acute response to ambulation may well be a neurogenic response, since neurogenic impulses to the kidney may cause an increase in tubular reabsorption of water and sodium. There is evidence to indicate that it is not of hormonal origin since Murphy and Stead (7) have shown that endogenous antidiuretic hormone does not increase sodium reabsorption in man. In spite of the apparent depression of excretion of water and electrolytes, none of the patients exhibited edema or other signs of fluid retention. In one patient studied by observations of excretion rates of water, sodium, potassium, and chloride during four-hour periods at which time he was either ambulatory or recumbent, there was a decrease in excretion during ambulation but an increase during recumbency. During the sleeping hours, excretion of sodium and water during the first (11 to 3 a.m.) recumbent period was greater than the second (3 to 7 a.m.) consecutive recumbent period, suggesting that during the first sleeping period there was an augmented excretion to compensate for the preceding period of depressed excretion. Although renal functions are depressed during ambulation, there is a return to the control values during recumbency even though the blood pressure is reduced during hexamethonium therapy. Thus, this period during recumbency may also allow for necessary homeostatic balance or compensation to prevent retention of renal excretion products such as urea nitrogen.

SUMMARY AND CONCLUSIONS

1. Thirteen patients with hypertension have been observed during oral hexamethonium therapy. Observations were made during recumbency as well as during ambulation.

2. In the supine position, there was a reduction in blood pressure after hexamethonium without a reduction in renal plasma flow, glomerular filtration rate, or tubular excretory capacity. These

observations indicate that the renal vessels in patients with hypertension are quite capable of dilating.

3. In the ambulatory state before hexamethonium, there was a depression of glomerular filtration, renal plasma flow, and TmPAH due to vasoconstriction. After hexamethonium therapy, the ambulatory state was accompanied by a further drop in blood pressure and a reduction in renal functions quite similar to those observed in the untreated patient but the changes were often more marked.

4. Ambulation after the administration of hexamethonium is accompanied by a decrease in the excretion of water, sodium, and potassium. This may be due to neurogenic impulses as suggested by the depression during ambulation alone without a drop in blood pressure.

5. Hexamethonium when used orally in the chronic treatment of hypertension appears to be acceptable since none of the changes described resulted in any significant alteration of their clinical status in terms of impaired excretion of nitrogenous substances or in the retention of sodium and water. Reactivity in the recumbent position seems to compensate for the depressed function during ambulation.

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AN INTRACARDIAC CATHETER WITH INFLATABLE BALLOON FOR PRODUCING INCREASED VENOUS PRESSURES IN THE VENAE CAVAE OF MAN[1]

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Pertinent to the problem of edema formation in congestive heart failure are the observations, largely in animals (1–5), which indicate that the renal excretions of water and electrolytes are decreased during induced venous congestion, either of the kidney alone or of venous areas including the kidneys. These observations suggested the desirability of studying the excretion of water and electrolytes in man in response to increased congestion and pressure in various parts of the venous system. In order to pursue such a study, a method was developed for producing controlled increases in venous pressure in the venae cavae, specifically in the superior vena cava and in the inferior vena cava at levels including and excluding the renal veins. This paper is a report of the method, which is based upon the use of a "balloon" catheter.

METHODS

A "balloon" catheter was constructed by fashioning a thin latex-rubber balloon about the proximal opening of a double lumen intracardiac catheter, while the distal opening was left open for the recording of pressures (Figure 1). The catheter is introduced into the superior or inferior vena cava of man by the technique of cardiac catheterization and positioned so that the balloon is in the desired location. The balloon is inflated with a dilute (20 per cent) solution of diodrast so that its location is readily determined by fluoroscopic examination (Figure 2). When the balloon was inflated in the inferior vena cava changes in distal venous pressure were measured in the femoral vein by a saline manometer and in the inferior vena cava, through the distal lumen of the catheter, by an optical recording system; when inflated in the superior vena cava, distal venous pressure was measured in the antecubital veins by a saline manometer.

As the pressure rises in the vena cava distal to the inflated balloon, the blood stream usually carries the balloon forward and often into the right atrium. When this occurs the elevated venous pressure in the distal veins promptly falls. An inflated balloon in the inferior vena cava is carried upward into the right atrium, with a great deal of slack catheter, and tends to move toward the tricuspid valve. On such occasions the balloon has been quickly deflated for fear that it might block completely the flow of blood. Such upward "riding" has been prevented by stiffening the catheter with a steel stylette, threaded into the proximal-lumen section of the catheter after the catheter has been positioned. The stylette only partially occupies the lumen and the diodrast solution is still readily introduced and withdrawn. In the superior vena cava the inflated balloon is also carried forward, but usually only to the extent of taking up the slack in the catheter. This may carry the balloon into the right atrium, but not as far as the tricuspid valve and stylettes are not required. Regardless of its location, the position of the balloon is repeatedly checked by fluoroscopy to determine that it remains in the desired position.

The balloon is inflated slowly, over the course of several minutes, to the extent required to produce the desired rise in distal venous pressure. Rapid inflation to a degree whereby most of the vena caval flow is suddenly obstructed can reduce the cardiac output so markedly that syncope may result. In the early observations, before this fact was fully realized, threatened syncope occurred in five subjects, as indicated by the onset of bradycardia, sweating, faintness, weakness and a fall in blood pressure, as low as 62/48 mm. Hg in one subject and 81/46 mm. Hg in another. In all instances

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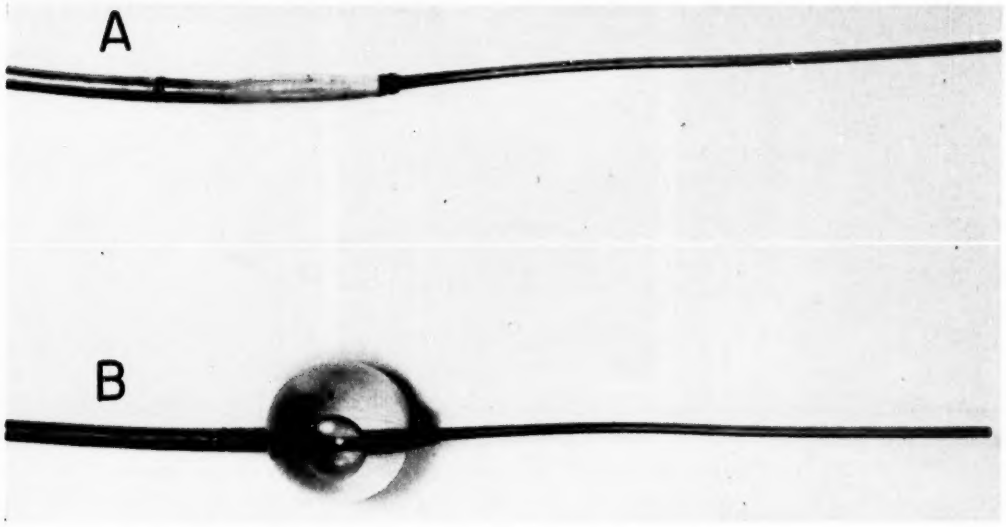

FIG. 1. PHOTOGRAPH OF DISTAL ONE-FOURTH OF "BALLOON" CATHETER

A. Balloon deflated and catheter ready for introduction into a peripheral vein. *B*. Balloon inflated with 8 ml. of 20 per cent diodrast solution. The circular highlight in the balloon is the air bubble formed by the air initially trapped in the lumen of the catheter.

the balloon was quickly deflated with prompt and apparently complete recovery. In several instances the balloon was then reinflated more slowly and distal venous pressures of 200 mm. saline were produced and maintained for 30 minutes without symptoms or change in arterial pressure.

In view of these experiences the procedure was adopted of inflating the balloon at a rate of 1 ml. per minute until the distal venous pressure reached 100 mm. saline and thereafter at a rate of 0.5 ml. per minute until the desired distal venous pressure, usually 170 to 250 mm. saline, was reached. Under such circumstances the arterial blood pressure and heart rate (Figure 3) remained virtually unchanged and no untoward symptoms appeared. Indeed, the subjects were unaware that anything had been done. Nevertheless, even when the balloon is inflated slowly, the blood pressure and heart rate should be determined repeatedly and the patient watched for evidence of circulatory distress. Upon the appearance of any untoward sign or symptom the balloon should be deflated immediately.

It quickly became apparent that the balloon had to be inflated to a considerable degree before the pressure rose in the distal veins. Thus, to obtain pressures in the range of 200 mm. saline in the inferior vena cava required the injection of 8 to 10 ml. of diodrast, when the balloon was above the renal veins, and 4 to 7 ml. when it was below the renal veins. Comparable pressures in the superior vena cava were obtained by the injection of 6 to 8 ml. of diodrast. At times, the inflated balloon was observed to occlude the vena cava entirely, as indicated by a change in shape of the balloon from a circular to a cylindrical outline and by a rise in distal venous pressure to 350 mm. saline or above. Such complete occlusions have been avoided and when recognized have been rectified immediately by deflating the balloon until it resumed its circular outline.

Rupture of the inflated balloon in a vein has not, thus far, occurred. In one subject the balloon was observed to deflate slowly after it had reached moderate size. The catheter was removed and on examination the balloon was found to have a "pin hole" leak, which became the site of rupture when the balloon was test-inflated rapidly and fully. This experience emphasizes the need for careful examination and test-inflation of the balloon before each use. The life of a balloon has proven to be short. After being used seven to ten times a

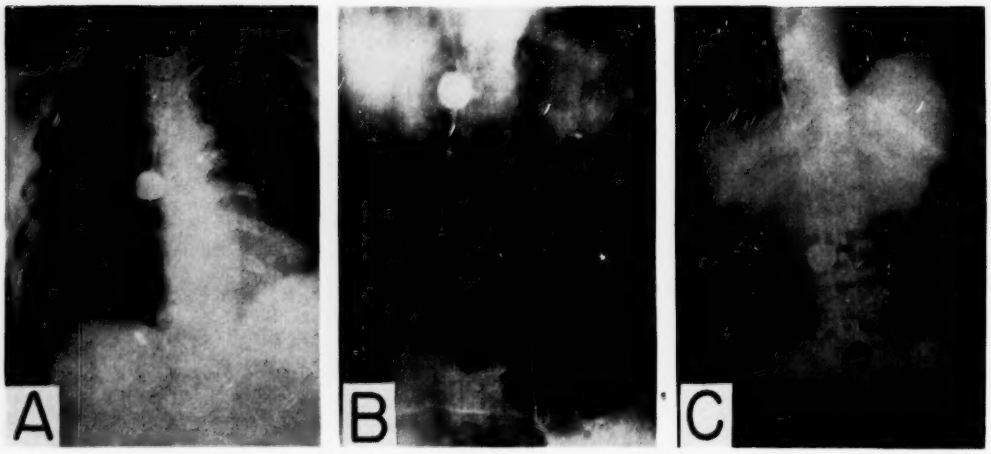

FIG. 2. X-RAYS SHOWING IDENTIFICATION AND POSITIONING OF THE INFLATED BALLOON

A. Superior vena cava. *B*. Inferior vena cava above the renal veins. *C*. Inferior vena cava below the renal veins.

balloon becomes worn and weakened and must be replaced.

The latex-rubber appears not to irritate the veins unduly. Local thrombosis of the peripheral vein of the arm has been the rule but only occasionally has it been of greater extent or severity than that seen after routine cardiac catheterization. One patient died from his primary disease one month after a study in which the balloon had been inflated in the inferior vena cava. On post-mortem examination the vena cava and its tributary veins were entirely normal.

OBSERVATIONS

The balloon has been inflated in the venae cavae of 49 patients without significant untoward effects. In the first nine subjects the period of inflation of the balloon was limited to 10 minutes. Subsequently, the duration of inflation was increased to a mean time of approximately 30 minutes and several intermittent inflations of such duration have been carried out over a two to four hour period in the same patient. The longest single period of inflation has been 50 minutes and the duration is being steadily and cautiously increased. In the last 43 observations the balloon has been inflated for periods of 20 to 40 minutes in 40 patients as follows: In the superior vena cava in 14 patients, in the inferior vena cava above the renal veins in 16

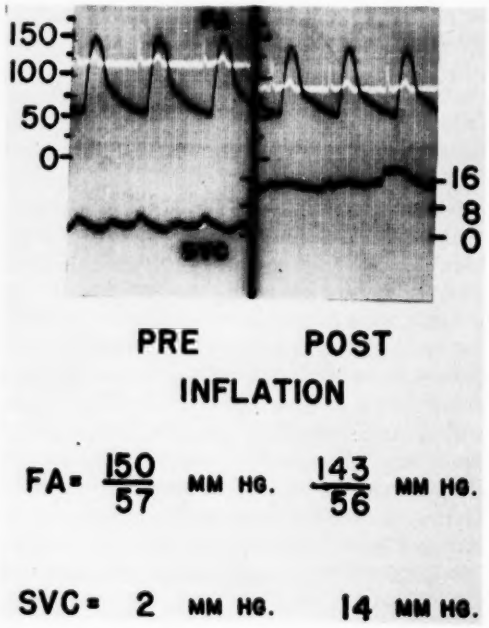

FIG. 3. FEMORAL ARTERIAL PRESSURE, SUPERIOR VENA CAVAL PRESSURE AND ELECTROCARDIOGRAM (LEAD II) BEFORE AND DURING INFLATION OF THE BALLOON IN THE SUPERIOR VENA CAVA

In this instance the balloon was about the distal opening, while the proximal opening was free for the measurement of pressures.

patients and in the inferior vena cava below the renal veins in 13 patients. The initial and final venous pressures, distal to the balloon, in these subjects are plotted in Figure 4.

COMMENT

Although venous congestion has, thus far, been produced only in the venae cavae, the application of the "balloon" catheter may be considerably wider. It seems quite feasible to use the method, or modifications of it, to produce venous congestion in any organ, the vascular tree of which is accessible to venous catheterization.

Details of construction of "balloon"

A "balloon" catheter of the type here described is manufactured by the United States Catheter and Instrument Corp. and has been used in animal studies (6). In our hands this catheter proved unsatisfactory in the larger veins of man, hence details of manufacture of the "balloon" catheter here used are described.

Preparation of catheter. A standard double lumen intracardiac catheter is scrupulously cleaned by soaking for 20 to 30 minutes in one liter of hot water containing 5 ml. of "Detergicide," [2] after which the catheter is rinsed in tap water and then distilled water. To prevent liquid latex from entering the catheter during dipping, the proximal opening is sealed with a small piece of parafilm and the distal opening is closed by hanging from the distal tip a small rubber bulb containing 2 to 3 ml. of mercury. The weighted bulb minimizes swaying of the catheter during dipping.

Dipping the catheter. The desired thickness of rubber and a firm fit of latex on the catheter is obtained by dipping the entire catheter, first in a saturated solution of calcium nitrate in absolute ethyl alcohol and then immediately in centrifuged latex.[3] In order to obtain a stronger layer of rubber around the proximal opening, the distal one-third of the catheter is re-dipped in the calcium nitrate solution. Finally, the entire length of the catheter is dipped in prevulcanized latex.[3] Centrifuged latex is used for its firm fit around the catheter, prevulcanized latex because it is "strong" and smooth when dry. The various dippings are carried out without pause or drying.

[2] Detergicide—a quaternary ammonium compound with high surface tension lowering properties, manufactured by U. S. Catheter & Instrument Corp., Glens Falls, N. Y.

[3] Generously supplied by Mr. H. Berger of Stein Hall & Co., New York City.

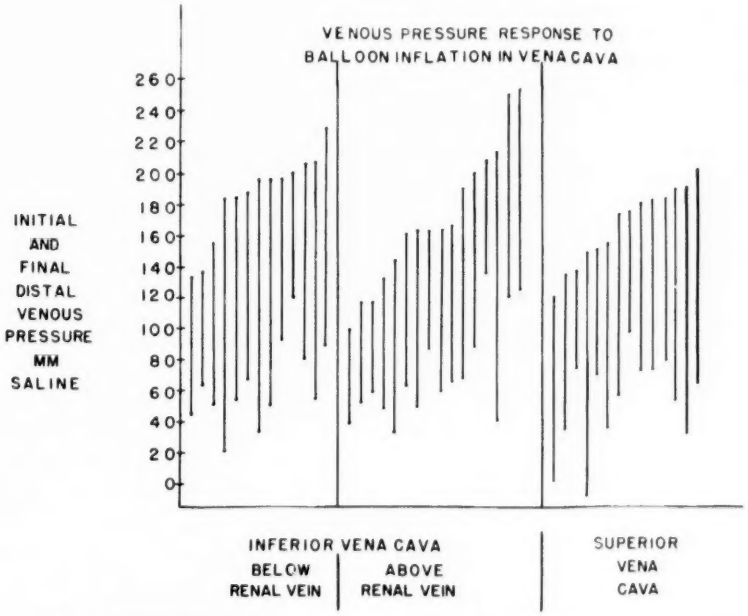

FIG. 4. INCREASE IN DISTAL VENOUS PRESSURES DURING INFLATION OF BALLOON IN THE SUPERIOR AND INFERIOR VENAE CAVAE OF FORTY SUBJECTS

Each vertical line connects the initial (lower point) and final (upper point) venous pressures of one subject. The designations along the abscissa indicate the site of the inflated balloon.

Drying and curing. The catheter is now dried in a warm box, heated with a 200 watt bulb, until the rubber is transparent. The latex is then cured by placing the catheter for 20 minutes in an electric oven at 90° C. In order to eliminate possible pyrogens in the rubber, the catheter with its rubber coating is soaked in cool 1N NaOH for one hour. It is then thoroughly washed in tap and distilled water and again dried in the warm box.

Tying the balloon. Temporary ties are applied above and below the proximal opening, and the rubber between the ties is distended by injecting water through the proximal lumen. An eccentric balloon forms. The balloon is deflated and the ties removed. The previously distended portion of the rubber is now pulled downward and fixed 1.5 cm. above and below the proximal opening by permanent ties of fine silk suture thread. The balloon when inflated is now symmetrical. The ties are fashioned by making a row of winds, pulling the free end under the winds, and securing it with a surgical knot. The rubber distal to the lower tie is cut and removed along with the rubber bulb, still hanging on the distal tip. The ties are then made more secure by applying to them a thin layer of varnish.

The balloon thus produced cannot slip off the catheter since the balloon is part of a rubber sheath which encases the entire catheter.[4] The capacity of the balloon is 8 ml. to 12 ml. of liquid and it inflates and deflates easily.

Sterilization. Sterilization is accomplished, at room temperature, by soaking the catheter and balloon, inside and out, in a solution of 5 ml. of "Detergicide" in a liter of distilled water. Wet heat weakens the rubber.

Stylette. In order to give rigidity to the catheter and prevent the balloon from "riding up" when it is in the inferior vena cava, a stylette of 0.020 inch stainless steel wire is used. It is inserted into the proximal-lumen section of the catheter until the proximal end of the wire is just flush with the hub of this lumen. Into the hub and over the wire end is then meshed a metal two-way stopcock which accommodates the syringe used to inject diodrast solution into the balloon. With the stopcock in the "off" position the inflation of the balloon is readily maintained.

[4] A recent development, used in 12 patients and *still on trial*, fashions a balloon about the proximal opening only and eliminates the rubber sheath encasing the rest of the catheter.

SUMMARY

A double lumen intracardiac catheter, equipped with a rubber balloon about the proximal opening, has been used to produce venous pressures up to 250 mm. saline in the superior and inferior venae cavae of man. This procedure has been performed on 49 patients without significant untoward effects. The balloon should be inflated slowly, for sudden, too rapid inflation to a marked degree may so obstruct venous return that syncope may result.

ACKNOWLEDGMENT

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ELECTROLYTE AND WATER EXCRETIONS AND RENAL HEMODYNAMICS DURING INDUCED CONGESTION OF THE SUPERIOR AND INFERIOR VENA CAVA OF MAN[1, 2]

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It is apparent that the kidney is the organ ultimately responsible for the salt and water retention, hence the edema formation, in congestive heart failure, but why the kidney fails to excrete these substances normally in this disease state remains to be determined. Venous congestion, and renal venous congestion in particular, has been postulated as one of the factors playing a role in the decreased electrolyte and water excretions. Recent observations in animals and man lend support to this concept.

In dogs, each of the following acute procedures, designed to elevate renal or systemic venous pressure or both, has induced a decrease in the urinary excretion of electrolytes and water: partial ligation of the inferior vena cava above the renal veins (1), induced pericardial effusion (2) and partial ligation of one renal vein (3, 4), a procedure which induced the effect on the side of the increased renal venous pressure but not on the contralateral normal side. In chronic experiments, inferior vena caval congestion, including the renal veins, did not cause a sustained retention of electrolytes and water, whereas constriction of the inferior vena cava above the diaphragm, which added congestion of the liver, led to sustained edema and ascites (1, 5).

In man, induced venous congestion has necessarily been of short duration. The renal excretion of electrolytes and water has been shown to decrease during quiet standing (6), during acute abdominal compression of sufficient degree to raise inferior vena caval pressure (7), and in association with acute congestion of the extremities produced by inflating cuffs about the thighs and arms (8). The opposite effect, an increase in water and salt excretion, has been reported during venous congestion of the head of sitting subjects (9), during ace bandaging of the legs (10), and during the fall in systemic venous pressure which occurs in patients with congestive heart failure as a result of the intravenous administration of digoxin (11).

These considerations suggested a study, in man, of the renal excretion of electrolytes and water in response to congestion of the venous system, so produced that the kidney could be included in, or excluded from, the congested area. Accordingly, venous congestion was produced at various levels of the inferior vena cava and in the superior vena cava by means of an inflatable balloon on an indwelling intracardiac catheter (12) and the resultant renal effects were determined. The data indicate that acute congestion of a sizable segment of either vena cava in man provokes a reduction in the urinary excretion of electrolytes and water.

METHODS AND PROCEDURES

General. The plan of study was to determine several renal, urinary, and cardiovascular functions before, during, and after induced vena caval congestion. Renal plasma flow and glomerular filtration rate were determined by clearance methods, using para-aminohippurate and inulin respectively. The urinary excretions of sodium, potassium, and chloride were measured in successive periods, and the concentrations of these electrolytes in the blood were determined periodically. Arterial blood pressure was measured directly by Hamilton manometers and optical recording. Peripheral venous pressure in the congested area (femoral or antecubital vein) was determined by saline manometer. When technically possible, vena caval pressure, either distal or proximal to the balloon, was recorded through the intracardiac catheter by Hamilton manometer. Heart rate was counted from the electrocardiogram.

Subjects. Convalescing hospitalized patients without overt cardiac or renal disease served as subjects. Most of

[1] This study was assisted by Grants-in-Aid from the Life Insurance Medical Research Fund and the New York Heart Association, Inc.

[2] Presented in part at the Forty-Third Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., April 30, 1951, and at the Twenty-Fifth Scientific Sessions of the American Heart Association, Cleveland, Ohio, April 19, 1952.

the subjects were in the older age group, hence arteriosclerosis and hypertension were frequently present. All patients received a regular hospital diet. Eight subjects, whose urinary sodium excretions were low, were given 6 to 8 grams of salt orally the day before the study, in order to assure a more adequate urinary excretion of sodium. No extra fluids were given immediately before or during the observations.

Procedures. The observations were made in the morning in the post-absorptive state and the following procedures were carried out routinely. An electrocardiogram was taken and an indwelling catheter was placed in the urinary bladder. After the injection of appropriate priming doses, a sustaining infusion of inulin and para-aminohippurate in distilled water was delivered continuously by a Bowman pump at a rate of 1 ml. per min. A "balloon" intracardiac catheter was placed in the desired location in the vena cava (12) and its distal lumen was perfused continuously with a slow drip of 5 per cent dextrose in water. A gauge No. 18 needle was inserted into the femoral or antecubital vein, attached to a saline manometer and intermittently flushed with small amounts of 0.9 per cent saline solution. The femoral artery was cannulated with an indwelling Cournand needle. Heparin, 50 to 75 mg. per L., was added to all infusions except that delivered by the pump. A 2 per cent solution of metycaine was used for local anesthesia.

Determinations were made in successive periods, each of 10 to 15 minutes duration. Arterial and vena caval pressures and the electrocardiogram were recorded at the mid-point of each period, while water and electrolyte excretions and renal hemodynamics were determined as averages for the period. All blood samples were of arterial blood and obtained at the mid-point of each period. Peripheral venous pressure in the congested area was read continuously at approximately two minute intervals. The zero point of reference for all pressure measurements was 5.5 to 6.5 cm. posterior to the angle of Louis.

After two to four control periods, the balloon was inflated slowly (over 6 to 10 minutes) with 20 per cent diodrast solution, injected at a rate of 0.5 to 1.0 ml. per min. until the distal venous pressure was raised to 150 mm. to 220 mm. saline. Then followed two to four periods during which the balloon was kept inflated and the distal venous pressure elevated. Thereafter, the balloon was deflated; the distal venous pressure fell promptly, and three to four additional periods of determinations were made. While the balloon was inflated its position was checked repeatedly by fluoroscopy to assure that it remained in the desired location: for the inferior vena cava above the renal veins, at or above the mid-point of the hepatic shadow and often just under the diaphragm; for the inferior vena cava below the renal veins, at or just above the iliac crests; for the superior vena cava, in the mid or lower third of the cava.

Analyses. Standard chemical methods were used for the determination of the blood and urine concentrations of inulin (13, 14), para-aminohippurate (15), and chloride (16). Sodium and potassium concentrations were determined by an internal standard flame photometer. Plasma, separated from erythrocytes within 15 minutes of sampling, was used for the determination of the above substances in blood except for sodium, which was determined in serum. Dry heparin was the anticoagulant. Hematocrits were obtained on arterial blood centrifuged in Wintrobe tubes. Plasma protein concentrations were derived from specific gravity measurements made by the copper sulfate technique (17). Systolic and diastolic arterial pressures were obtained by averaging the respective pressures over two respiratory cycles; mean arterial pressure and mean vena caval pressure were determined by planimetric integration of these two pressure waves over the same two respirations. The average of the saline manometer readings during a period was taken as the representative peripheral venous pressure for the period.

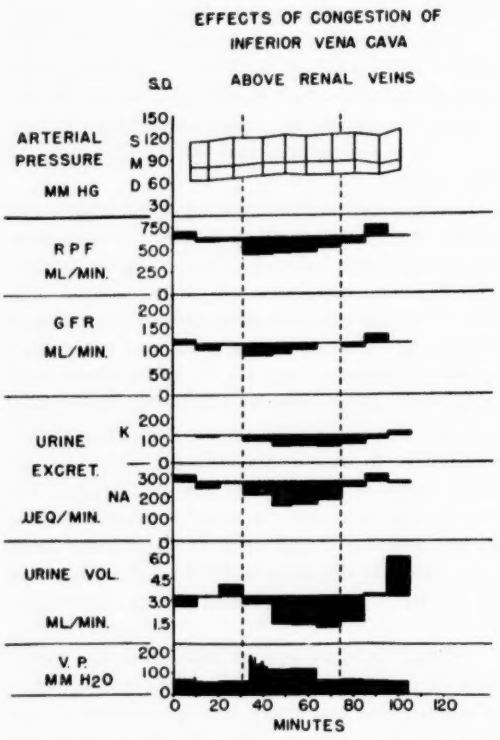

FIG. 1. THE EFFECTS OF INFERIOR VENA CAVAL CONGESTION, INCLUDING THE RENAL VEINS; A TYPICAL RESPONSE

The dotted line on the left indicates inflation of balloon in the inferior vena cava above the renal veins, the dotted line on the right, deflation of the balloon.

Systemic arterial pressures and peripheral venous pressures are plotted as determined values above zero; water and electrolyte excretions, glomerular filtration rates and renal plasma flows are plotted, for each period, as changes from the average of their respective three control determinations.

RESULTS

With few exceptions, the subjects tolerated satisfactorily the confining and tedious three to four hour study. While the balloon was inflated they were unaware of anything unusual, offered no complaints, and showed no evidence of circulatory distress. This benign state was attributed to the slow inflation of the balloon and to keeping the elevated distal venous pressure under 250 mm. saline. In several initial observations, impending syncope resulted when the balloon was inflated too rapidly and the distal venous pressure was raised to higher levels. The data of these observations have been excluded from this report.

The *pattern* of the changes produced by vena caval congestion was largely the same whether the increase in venous pressure involved the inferior vena cava including the renal veins (Figure 1), the inferior vena cava below the renal veins (Figure 2), or the superior vena cava (Figure 3). For this reason, the effects of congestion of one area only, that produced by inflating the balloon in the inferior vena cava above the renal veins, will be described in detail. It is to be understood that the effects of congestion of the other two areas were similar, except for the specific differences which will be presented.

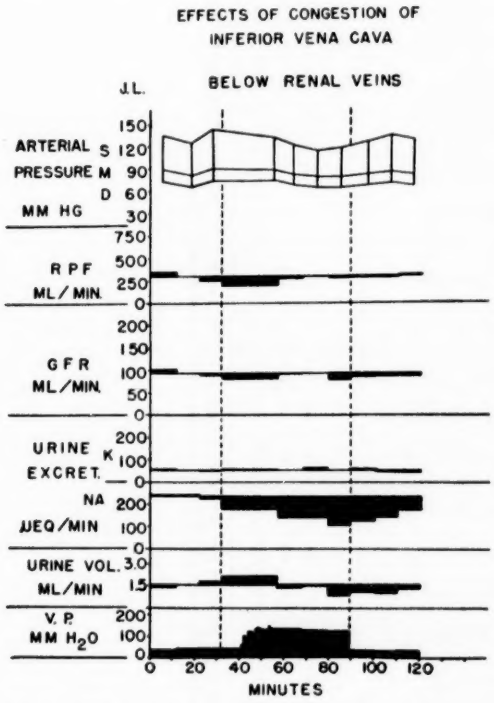

FIG. 2. THE EFFECTS OF INFERIOR VENA CAVAL CONGESTION BELOW THE RENAL VEINS; A TYPICAL RESPONSE

The dotted line on the left indicates inflation of balloon in the inferior vena cava below the renal veins, the dotted line on the right, deflation of the balloon.

For details of plotting see Figure 1.

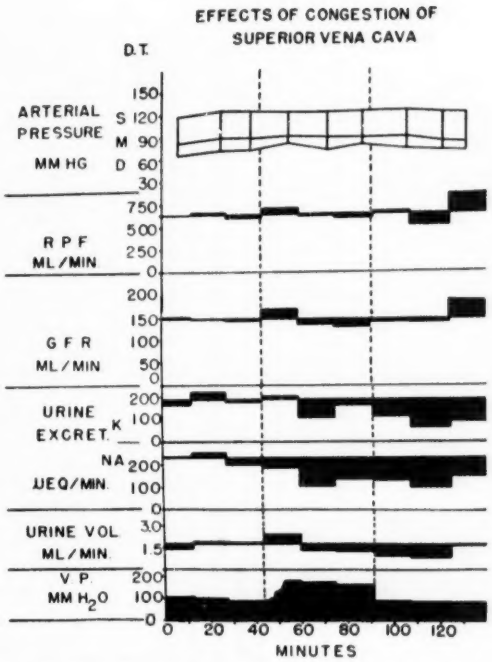

FIG. 3. THE EFFECTS OF SUPERIOR VENA CAVAL CONGESTION; A TYPICAL RESPONSE

The left dotted line indicates inflation of balloon in lower third of the superior vena cava, the right dotted line, deflation of the balloon.

For details of plotting see Figure 1.

Tables I to VIII present the data and their statistical analysis by the method of the *critical ratio* (*t* test). The analysis was made for each control, inflation, and post-deflation period for each function measured. For each period only the data for the subjects represented in that period were used and the values for each individual were always analyzed with respect to the *average of his control period values*. Hereafter, the words "significant" will be used only where the P value is 0.01 or less, "probably significant" where the P

TABLE I

Effect of vena caval congestion on water excretion
(Data are urine flows in ml./min.)

Position of balloon	Inferior vena cava, above renal veins										Inferior vena cava, below renal veins										Superior vena cava								
Status of balloon	Before inflation			During inflation			After deflation				Before inflation			During inflation			After deflation				Before inflation			During inflation			After deflation		
Period	1	2	3	1	2	3	1	2	3		1	2	3	1	2	3	1	2	3		1	2	3	1	2	3	1	2	3
S. D.	2.6	3.2	4.0	2.8	1.4	1.4	1.2	1.6	3.5	S. D.	1.6	3.5	6.0	8.1	2.9	0.9	1.2	0.9	—	J. T.	2.4	1.9	2.4	2.5	1.2	1.2	1.0	1.3	1.5
C. Y.	1.0	0.9	1.0	0.7	0.7	0.5	0.6	0.7	0.7	C. Y.	0.6	0.7	0.7	0.7	0.8	0.7	0.9	—	—	T. A.	0.6	0.6	1.0	1.2	1.4	0.7	0.6	0.4	0.8
J. L.	1.1	1.0	1.4	1.1	0.9	0.1	0.9	1.2	—	J. L.	1.4	1.5	1.8	2.1	1.3	1.4	1.1	1.0	1.4	G. T.	1.9	2.2	4.2	5.7	0.9	1.3	1.7	1.4	1.5
M. F.	0.7	0.7	0.9	0.8	0.4	—	0.3	0.6	0.8	M. F.	0.6	0.8	0.8	1.8	2.0	—	1.3	1.7	—	J. S.	0.8	0.6	0.6	0.5	0.4	0.3	0.5	0.5	0.5
J. K.	2.7	—	2.3	2.8	2.2	—	3.6	5.5	—	J. K.	1.9	1.2	3.2	4.0	5.8	3.6	2.7	—	2.3	H. H.	2.7	3.0	4.7	4.7	1.8	1.2	1.0	1.1	1.4
G. B.	1.0	1.2	1.2	1.1	0.7	0.7	0.7	1.3	1.1	G. B.	1.1	1.1	1.4	1.1	1.1	0.7	1.0	1.2	1.2	A. W.	1.4	0.9	1.2	0.8	1.0	1.3	0.5	1.2	0.7
J. P.	—	1.2	1.3	1.0	1.0	0.6	1.3	1.4	1.3	J. P.	1.8	0.8	1.1	0.9	0.8	1.0	0.8	1.1	1.2	F. G.	2.1	2.9	4.8	4.6	5.8	3.5	2.0	2.8	3.8
S. D. M.	0.9	0.9	0.8	0.9	0.7	0.6	0.7	0.8	1.0	S. D. M.	1.0	0.9	1.0	1.0	0.8	0.7	0.7	0.9	0.9	F. S.	1.4	1.5	1.2	1.2	1.3	0.8	1.1	0.8	0.8
T. H.	3.4	3.3	3.1	2.2	1.2	0.9	1.0	1.3	1.2	J. D.	1.1	1.1	1.1	1.1	0.7	0.7	0.8	0.8	1.0	D. T.	1.5	1.9	1.9	2.4	1.4	1.3	1.0	0.9	1.7
L. W.	—	1.0	0.6	0.6	0.5	0.5	0.7	—	0.6	T. F.	4.2	6.9	6.8	4.4	3.0	0.1	1.6	1.2	1.1	H. C.	2.1	5.0	8.2	6.9	2.4	1.6	1.9	2.5	3.1
R. D.	4.1	4.4	5.6	3.2	1.2	0.7	0.5	0.9	—	A. C.	1.5	1.6	1.3	1.1	1.3	1.3	1.1	1.4	—	J. P.	1.3	1.0	1.1	0.2	1.4	1.2	0.9	0.7	0.9
M. K.	0.9	1.6	1.5	1.7	1.5	1.2	1.5	1.0	1.5	B. McK.	0.6	0.6	0.6	0.5	0.5	—	0.6	—	—	L. C.	0.9	1.0	1.2	0.8	0.6	0.4	0.6	0.5	0.7
E. H.	0.5	0.5	0.5	0.2	0.2	0.2	0.3	0.4	0.3	P. M.	1.2	1.2	1.6	1.5	0.7	0.7	0.8	0.5	0.7										
R. C.	—	0.9	0.8	0.8	0.7	0.3	0.6	0.9	0.9																				
A. B.	2.8	4.2	3.5	1.4	0.5	—	0.6	—	—																				
J. R.	—	1.4	2.2	2.1	1.4	—	2.0	2.5	2.2																				
Number of subjects per period, N	12	15	16	16	16	12	16	14	12		13	13	13	13	13	11	13	10	8		12	12	12	12	12	12	12	12	12
Mean value per period, N subjects	1.8	1.8	1.9	1.4	0.9	0.6	1.0	1.4	1.3		1.4	1.7	2.1	2.2	1.7	1.1	1.1	1.1	1.2		1.6	1.9	2.7	2.6	1.6	1.2	1.1	1.2	1.4
Mean control value for N subjects	2.0	1.8	1.8	1.8	1.8	1.7	1.8	1.8	1.4		1.7	1.7	1.7	1.7	1.7	1.9	1.7	1.9	1.9		2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
P*	0.1	>0.8	0.3	<0.01	<0.01	<0.01	0.04	0.4	0.3		0.2	>0.8	>0.8	0.3	>0.8	0.2	0.1	0.2	0.3		0.2	0.1	0.03	0.07	0.3	0.05	<0.01	<0.01	0.04

* P—Statistical probability per period determined by the method of *critical ratio* (*t* test). For each period the analysis is made by comparing the values for each of the subjects in that period with the *average of his control period values.*

TABLE II

Effect of vena caval congestion on urinary sodium excretion
(Data are sodium excretions in μEq./min.)

Position of balloon	Inferior vena cava, above renal veins										Inferior vena cava, below renal veins										Superior vena cava								
Status of balloon...	Before inflation			During inflation			After deflation				Before inflation			During inflation			After deflation				Before inflation			During inflation			After deflation		
Period.....	1	2	3	1	2	3	1	2	3		1	2	3	1	2	3	1	2	3		1	2	3	1	2	3	1	2	3
S. D.	298	240	262	206	157	163	186	243	301	S. D.	243	301	258	298	147	136	229	203	—	J. T.	201	171	162	140	80	122	120	139	130
C. Y.	190	189	216	152	128	84	86	104	121	C. Y.	86	104	121	126	127	130	161	—	—	T. A.	66	71	77	47	45	45	49	48	56
J. L.	129	144	178	147	88	17	116	150	—	J. L.	242	242	222	176	140	141	129	144	178	G. T.	208	184	202	168	95	124	172	159	157
M. F.	127	113	134	89	52	—	49	85	111	M. F.	85	111	105	134	111	—	96	145	—	J. S.	76	54	42	41	29	25	40	42	45
J. K.	—	—	63	123	48	—	61	91	—	J. K.	—	—	91	64	62	33	43	—	86	H. H.	398	368	452	341	233	205	235	238	273
G. B.	101	128	146	123	86	65	76	141	139	G. B.	138	129	145	139	115	70	101	128	146	A. W.	175	153	216	174	155	169	71	190	140
J. P.	—	106	102	84	71	58	100	104	102	J. P.	100	61	78	70	64	60	67	92	98	F. G.	54	59	69	57	48	33	36	54	72
S. D. M.	149	157	118	135	111	81	105	126	180	S. D. M.	183	151	173	148	115	96	97	149	157	F. S.	194	196	163	155	144	86	105	79	84
T. H.	193	271	258	202	132	123	141	161	141	J. D.	182	188	177	154	107	111	120	118	136	D. T.	238	254	200	186	106	137	131	102	154
L. W.	—	110	88	76	54	51	66	—	77	T. F.	159	179	130	97	172	44	118	155	164	H. C.	196	177	218	182	182	204	225	248	210
R. D.	224	195	230	130	55	69	59	104	—	A. C.	101	107	92	76	72	97	101	99	—	J. P.	151	147	164	76	153	134	105	95	100
M. K.	123	171	226	202	160	149	170	118	204	B. McK.	17	17	19	11	7	—	12	—	—	L. C.	142	177	150	123	87	48	78	69	101
E. H.	35	42	48	33	18	12	11	19	19	P. M.	131	105	127	98	69	84	76	62	64										
R. C.	—	103	106	98	81	57	87	105	114																				
A. B.	113	65	88	58	30	—	43	—	—																				
J. R.	—	195	331	303	181	—	279	375	319																				
Number of subjects per period, N	11	15	16	16	16	12	16	14	12		12	12	13	13	13	11	13	10	8		12	12	12	12	12	12	12	12	12
Mean value per period, N subjects	153	149	162	135	91	77	102	138	152		139	141	134	122	105	91	104	130	129		175	168	167	141	113	111	114	122	127
Mean control value for N subjects	162	157	151	151	151	156	151	159	158		139	139	135	135	135	149	135	155	146		170	170	170	170	170	170	170	170	170
P*	0.4	0.2	0.05	0.1	<0.01	<0.01	<0.01	0.2	0.7		>0.8	0.6	0.6	0.1	0.01	<0.01	0.02	0.1	0.2		0.3	0.5	0.4	<0.01	<0.01	<0.01	<0.01	0.02	<0.01

* P—Statistical probability per period determined by the method of *critical ratio* (*t* test).

TABLE III

Effect of vena caval congestion on urinary potassium excretion
(The data are averaged potassium excretions in μEq./min. for number of subjects indicated)

Position of balloon	Inferior vena cava, above renal veins								
Status of balloon	Before inflation			During inflation			After deflation		
Period	1	2	3	1	2	3	1	2	3
Number of subjects per period, N	11	15	16	16	16	12	16	14	12
Mean value per period, N subjects	66	58	57	52	42	42	54	.61	52
Mean control value for N subjects	64	59	58	58	58	60	58	55	53
P*	0.4	0.4	0.5	0.2	<0.01	<0.01	0.4	>0.8	0.8
Position of balloon	Inferior vena cava, below renal veins								
Number of subjects per period, N	11	11	13	13	13	11	13	10	8
Mean value per period, N subjects	59	58	54	52	50	42	53	52	54
Mean control value for N subjects	58	58	55	55	55	56	55	56	56
P*	0.7	>0.8	>0.8	0.3	0.3	0.02	0.7	0.6	0.6
Position of balloon	Superior vena cava								
Number of subjects per period, N	12	12	12	12	12	12	12	12	12
Mean value per period, N subjects	81	90	96	85	73	70	69	63	63
Mean control value for N subjects	88	88	88	88	88	88	88	88	88
P*	0.05	0.5	0.08	0.4	<0.01	<0.01	<0.01	0.02	<0.01

* P—Statistical probability per period determined by the method of *critical ratio* (*t* test).

value is 0.02 or 0.03, and "possibly significant" for P values of 0.04 and 0.05.

I. *Effects of inflation of the balloon in the inferior vena cava above the renal veins*

Water excretion (*Table I*). During the venous congestion, urine flow decreased in all 16 subjects, moderately to markedly in 13, slightly in two, equivocally in one. This effect occurred at varying rates of control urine excretion between 0.5 ml. per min. (E. H.) and 4.7 ml. per min. (R. D.). The decrease was usually progressive, and after the first period of congestion urine flow was significantly less than the control excretion. Following deflation of the balloon, urine flow increased in all subjects, and 30 minutes later reached control levels in 11 of the 16 subjects. Twenty to thirty minutes after the deflation the flow rates were not significantly different from control values.

Albuminuria did not occur, either during the venous congestion or after its release.

Sodium excretion (*Table II*). Sodium excretion decreased moderately to markedly in all 16 subjects. Again the effect was usually progressive, and after the tenth minute of congestion, sodium excretion fell significantly below control values. The decrease occurred at both low and high levels of control excretion; thus, from 42 μEq. per min. to 12 μEq. per min. in E. H. and from 267 μEq. per min. to 163 μEq. per min. in S. D. Following deflation of the balloon the excretion rate rose in all subjects. During the first post-deflation period the rate was still significantly reduced, but thereafter sodium excretion was not significantly different from control values.

Potassium excretion (*Table III*). Potassium excretion decreased less consistently than did water or sodium excretion. The decreases tended to be less progressive and apparently greater at the higher levels (R. D., 124 μEq. per min. to 80 μEq. per min.) than the lower levels (R. C., 19 μEq. per min. to 16 μEq. per min.) of control excretion. Nevertheless, for the group, potassium excretion

was significantly reduced during the second and third periods of congestion. After release of congestion the excretion rates returned promptly to control levels.

Chloride excretion (*Table IV*). Chloride excretion decreased in a manner similar to sodium excretion. Moderate to marked falls occurred at both high (S. D., 330 μEq. per min. to 203 μEq. per min.) and low (T. H., 24 μEq. per min. to 15 μEq. per min.) levels of control excretion in 15 of the 16 subjects, and the mean excretion rate was significantly reduced during the second and third periods of congestion. With release of congestion, chloride excretion usually increased promptly but still remained significantly reduced during the first post-deflation period, after which control values were reached.

Renal plasma flow (*Table V*). Probably due to advanced age of the subjects and the coexistence of hypertension and arteriosclerosis, the mean control renal plasma flow was low (348 ml. per min.). A variable decrease in flow, averaging 20 to 30 per cent, occurred during at least one of the periods of congestion in each of the eight subjects studied. As the venous congestion was maintained, the flow rates varied and did not change progressively as in the case of the electrolyte excretions. For the group, renal plasma flow was significantly decreased during the second period of congestion and probably significantly reduced during the third. Following deflation of the balloon, renal plasma flow returned promptly to control values.

Glomerular filtration rate (*Table VI*). The mean control filtration rate was somewhat reduced (90 ml. per min.), again probably a function of the type of subjects. A variable (10 to 25 per cent), usually small, decrease in filtration rate occurred during the first two periods of congestion in all eight subjects studied and at both normal (R. D., 133 ml. per min. to 82 ml. per min.) and impaired (M. K., 44 ml. per min. to 34 ml. per min.) filtration rates. In the third congestion period, filtration rate rose in five subjects and in three reached control levels, but the mean value for the

TABLE IV

Effect of vena caval congestion on urinary chloride excretion
(The data are averaged chloride excretions in μEq./min. for number of subjects indicated)

Position of balloon	Inferior vena cava, above renal veins								
Status of balloon	Before inflation			During inflation			After deflation		
Period	1	2	3	1	2	3	1	2	3
Number of subjects per period, N	12	15	16	16	16	12	16	14	12
Mean value per period, N subjects	150	152	164	151	101	94	117	146	158
Mean control value for N subjects	154	160	154	154	154	149	154	158	159
P*	0.6	0.2	0.1	0.7	<0.01	<0.01	<0.01	0.4	>0.8
Position of balloon	Inferior vena cava, below renal veins								
Number of subjects per period, N	13	13	13	13	13	11	13	10	8
Mean value per period, N subjects	168	153	154	157	131	106	121	156	156
Mean control value for N subjects	158	158	158	158	158	166	158	171	169
P*	0.3	0.5	0.3	>0.8	0.02	<0.01	<0.01	0.2	0.2
Position of balloon	Superior vena cava								
Number of subjects per period, N	12	12	12	12	12	12	12	12	12
Mean value per period, N subjects	199	195	222	166	124	130	134	136	140
Mean control value for N subjects	202	202	202	202	202	202	202	202	202
P*	0.7	0.4	0.06	<0.01	<0.01	<0.01	<0.01	0.02	0.02

* P—Statistical probability per period determined by the method of *critical ratio* (*t* test).

TABLE V

Effect of vena caval congestion on renal plasma flow
(Data are renal plasma flows in ml./min.)

Position of balloon	Inferior vena cava, above renal veins										Inferior vena cava, below renal veins										Superior vena cava								
Status of balloon	Before inflation			During inflation			After deflation				Before inflation			During inflation			After deflation				Before inflation			During inflation			After deflation		
Period	1	2	3	1	2	3	1	2	3		1	2	3	1	2	3	1	2	3		1	2	3	1	2	3	1	2	3
S. D.	703	590	600	444	455	462	510	568	765	S. D.	568	765	650	561	329	340	600	540	—	J. T.	651	556	528	469	292	593	523	430	468
C. Y.	233	249	301	242	244	209	299	319	317	C. Y.	299	319	317	304	354	270	479	—	—	T. A.	332	350	400	356	370	333	325	296	354
J. L.	284	283	312	230	260	42	450	324	—	J. L.	354	303	248	198	272	299	284	283	312	G. T.	430	415	488	426	263	392	492	420	446
T. H.	283	322	293	304	201	211	252	241	248	J. D.	368	362	397	480	284	332	345	338	405	J. S.	275	258	271	288	263	223	344	318	376
L. W.	—	342	326	286	256	310	367	—	370	T. F.	750	664	565	398	790	230	660	820	655	H. H.	527	608	650	420	395	401	513	528	620
R. D.	424	393	409	321	218	335	329	420	—	A. C.	360	348	—	296	288	412	444	375	—	A. W.	386	247	287	375	334	373	196	429	303
M. K.	98	142	193	144	142	119	153	110	172	B. McK.	333	324	338	282	177	—	341	—	—	F. G.	310	311	338	290	318	259	200	226	271
E. H.	329	427	420	378	250	426	478	476	420	P. M.	462	382	420	382	333	394	388	343	404	F. S.	473	338	303	554	452	333	526	554	469
																				D. T.	636	670	610	710	625	600	660	516	850
																				H. C.	395	388	434	288	381	400	482	482	435
																				J. P.	371	426	483	229	487	452	377	319	334
																				L. C.	323	359	367	339	229	270	421	352	426
Number of subjects per period, N	7	8	8	8	8	8	8	7	6		8	8	7	8	8	7	8	6	4		12	12	12	12	12	12	12	12	12
Mean value per period, N subjects	336	344	357	294	253	264	355	351	382		437	433	419	363	353	325	443	445	444		426	410	430	395	366	386	413	406	446
Mean control value for N subjects	350	348	348	348	348	348	348	350	345		426	426	436	426	426	433	426	431	440		417	417	417	417	417	417	417	417	417
*P	0.2	0.6	0.3	0.04	<0.01	0.03	>0.8	>0.8	0.19		0.6	0.6	0.3	0.1	0.2	0.2	0.6	0.7	0.7		0.1	0.4	0.3	0.5	0.7	0.2	0.8	0.7	0.3

* P—Statistical probability per period determined by the method of *critical ratio* (*t* test).

TABLE VI

Effect of vena caval congestion on glomerular filtration rate
(Data are filtration rates in ml./min.)

Position of balloon	Inferior vena cava, above renal veins										Inferior vena cava, below renal veins										Superior vena cava								
Status of balloon	Before inflation			During inflation			After deflation				Before inflation			During inflation			After deflation				Before inflation			During inflation			After deflation		
Period	1	2	3	1	2	3	1	2	3		1	2	3	1	2	3	1	2	3		1	2	3	1	2	3	1	2	3
S. D.	126	103	113	88	92	104	114	108	133	S. D.	108	133	117	106	71	96	112	85	—	J. T.	139	133	123	116	96	148	124	126	128
C. Y.	77	76	92	76	80	70	87	88	85	C. Y.	87	88	85	86	97	81	104	—	—	T. A.	75	80	88	82	86	81	80	72	88
J. L.	86	86	87	76	84	23	114	94	—	J. L.	102	94	88	82	92	94	86	86	87	G. T.	102	110	113	106	74	107	136	106	113
T. H.	82	93	90	82	70	79	82	84	78	J. D.	93	90	91	100	85	82	84	76	96	J. S.	83	75	61	73	74	64	82	84	84
L. W.	—	77	66	60	55	63	73	—	72	T. F.	107	94	82	63	141	39	93	137	108	H. H.	132	116	137	110	98	98	115	136	135
R. D.	145	125	129	104	82	122	114	128	—	A. C.	89	88	82	70	68	98	86	82	—	A. W.	104	87	124	89	102	112	52	86	81
M. K.	27	40	49	43	39	34	43	38	49	B. McK.	90	82	81	70	51	—	102	—	—	F. G.	71	74	76	74	76	74	73	70	71
E. H.	88	99	102	85	61	97	112	117	92	P. M.	95	—	94	83	77	93	95	72	78	F. S.	108	125	90	140	139	94	106	148	105
																				D. T.	154	148	143	171	134	128	139	138	184
																				H. C.	84	83	79	90	86	92	102	95	78
																				J. P.	82	85	104	48	108	104	86	70	68
																				L. C.	72	88	96	92	103	74	117	92	101
Number of subjects per period, N	7	8	8	8	8	8	8	7	6		8	7	8	8	8	7	8	6	4		12	12	12	12	12	12	12	12	12
Mean value per period, N subjects	90	87	91	77	70	74	92	94	85		96	96	90	83	85	83	95	90	92		101	100	103	99	98	98	101	102	101
Mean control value for N subjects	93	90	90	90	90	90	90	93	84		94	94	94	94	94	95	94	97	94		100	100	100	100	100	100	100	100	100
P*	0.6	>0.8	0.6	<0.01	0.01	0.04	0.7	0.8	>0.8		0.3	0.2	0.08	0.04	0.4	0.2	0.7	0.6	>0.8		>0.8	>0.8	0.4	>0.8	0.8	0.7	>0.8	0.4	0.4

* P—Statistical probability per period determined by the method of *critical ratio* (*t* test).

TABLE VII

*Effect of vena caval congestion on systemic arterial blood pressures**
(The data are averaged blood pressures in mm. Hg per period for number of subjects indicated)

Position of balloon	Inferior vena cava above renal veins			Inferior vena cava below renal veins			Superior vena cava		
Status of balloon	During inflation			During inflation			During inflation		
Period	1	2	3	1	2	3	1	2	3
Number of subjects per period, N	8	9	9	8	8	8	12	12	11
Systolic blood pressure									
Mean value per period, N subjects	153	143	145	141	139	138	142	142	139
Mean control value for N subjects	155	152	152	149	149	148	147	147	146
P†	0.7	0.1	0.4	<0.01	0.08	0.03	0.02	0.3	0.05
Diastolic blood pressure									
Mean value per period, N subjects	78	74	76	74	72	73	76	76	76
Mean control value for N subjects	76	75	75	73	73	73	75	75	76
P†	0.3	>0.8	>0.8	0.6	0.6	—	0.2	0.2	—
Mean blood pressure									
Mean value per period, N subjects	96	94	91	91	89	90	97	100	97
Mean control value for N subjects	96	95	95	93	93	93	99	99	99
P†	—	>0.8	0.2	0.05	0.2	0.3	0.06	0.7	0.02
Pulse pressure									
Mean value per period, N subjects	75	70	71	68	66	64	66	66	63
Mean control value for N subjects	80	79	79	76	76	75	72	72	70
P†	0.1	0.04	0.2	<0.01	0.02	0.02	<0.01	<0.01	0.01

* The variations in arterial pressures from period to period in each subject were small, therefore the individual data have been omitted. The data for the 3 before inflation and 3 after deflation periods have been omitted, since in no instance did the value in any of these periods differ significantly from the average of the control period values.
† P—Statistical probability per period determined by the method of *critical ratio* (*t* test).

group was still decreased. Statistically, the decreases during the first and second periods of congestion were significant and during the third period possibly significant. Upon deflation of the balloon, filtration rates promptly rose to control levels.

Filtration fraction changed but little and not significantly. The average of the control values was 0.25. The mean values were 0.26, 0.28, and 0.24 respectively, for the three periods of venous congestion, and 0.26, 0.26, and 0.24 for the three successive post-deflation periods.

Arterial blood pressure (*Table VII*). In individual subjects the changes in arterial pressure varied from a fall (E. H., 149/60 to 118/52 mm. Hg) to no change (S. D., 116/63 to 121/68 mm. Hg) to a rise (R. D., 196/91 to 216/116 mm. Hg). Whatever the change, it was almost always small. The group response consisted of a slight fall (-7 to -9 mm. Hg) in systolic pressure with no change or a slight rise (-1 to $+2$ mm. Hg) in diastolic pressure. As a result, pulse pressure usually narrowed slightly (-5 to -9 mm. Hg) while mean pressure remained unchanged or fell slightly (-1 to -4 mm. Hg). Only the fall in pulse pressure in the second inflation period approached a significant change ($P = 0.04$).

Neither the heart rate (Table VIII) nor the electrocardiogram changed significantly at any time.

The arterial hematocrit and plasma protein concentration remained unchanged during and after the venous congestion, hence significant hemoconcentration probably did not occur.

II. *Effects of inflation of the balloon in the inferior vena cava below the renal veins*

The water, electrolyte, and renal hemodynamic changes produced by inferior vena caval congestion below the renal veins differed from the changes during vena caval congestion including the kidney in one respect, namely, that the effects were considerably more variable. In individual subjects the various functions changed just as markedly as during vena caval congestion including the kidney, but in other instances there were no changes and occasionally increases occurred. As a consequence, the changes were often less significant, although almost always of the type already described.

During the venous congestion, urine flow (Table I) decreased in only 8 of 13 subjects, remained unchanged in three, and increased in two. The mean value decreased, but not significantly. This variability persisted after release of congestion; urine flow rose in six subjects, remained unchanged in four, and decreased further in three. The mean value returned toward, but not to, the control level.

Sodium excretion (Table II) decreased significantly in the second and third periods of congestion. However, a decided fall occurred in only nine subjects, no change in three, and an increase in the remaining subject. The changes in potassium excretion (Table III) were even more variable but there still was a probably significant decrease in excretion during the third period of congestion. Chloride excretion (Table IV) again decreased in a manner similar to sodium excretion. After deflation of the balloon, the excretions of the three electrolytes and water returned to or toward control levels, but significant decreases in chloride excretion and probably significant reductions in sodium excretion persisted through the first post-deflation period.

Renal plasma flow (Table V) decreased definitely in only four of eight subjects and the mean flow rate did not change significantly. However, the magnitude of the decrease in the mean plasma flow (− 25 per cent) was not less than the decrease (− 27 per cent) which occurred when the renal veins were included in the congested area. Only the variability was greater. Glomerular filtration rate (Table VI) decreased slightly in six of eight subjects during the first 10 minutes of congestion, and the decrease was possibly signifi-

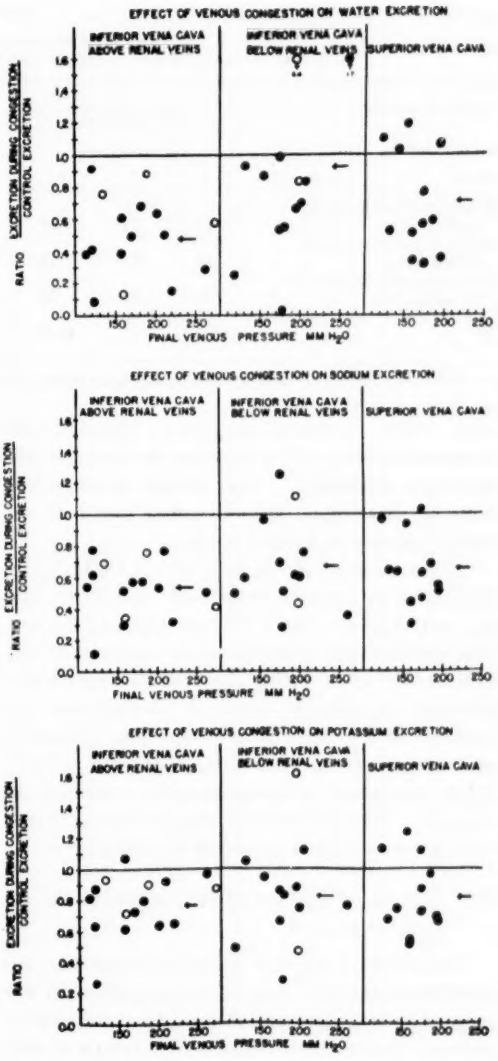
EFFECT OF VENOUS CONGESTION ON WATER EXCRETION
INFERIOR VENA CAVA ABOVE RENAL VEINS
INFERIOR VENA CAVA BELOW RENAL VEINS
SUPERIOR VENA CAVA
RATIO EXCRETION DURING CONGESTION / CONTROL EXCRETION
FINAL VENOUS PRESSURE MM H2O
EFFECT OF VENOUS CONGESTION ON SODIUM EXCRETION
INFERIOR VENA CAVA ABOVE RENAL VEINS
INFERIOR VENA CAVA BELOW RENAL VEINS
SUPERIOR VENA CAVA
RATIO EXCRETION DURING CONGESTION / CONTROL EXCRETION
FINAL VENOUS PRESSURE MM H2O
EFFECT OF VENOUS CONGESTION ON POTASSIUM EXCRETION
INFERIOR VENA CAVA ABOVE RENAL VEINS
INFERIOR VENA CAVA BELOW RENAL VEINS
SUPERIOR VENA CAVA
RATIO EXCRETION DURING CONGESTION / CONTROL EXCRETION
FINAL VENOUS PRESSURE MM H2O

FIG. 4. RELATIONSHIP BETWEEN THE DECREASES IN WATER, SODIUM, AND POTASSIUM EXCRETIONS AND THE DEGREE OF VENOUS CONGESTION OF THE INFERIOR AND SUPERIOR VENAE CAVAE

The abscissae indicate the elevated distal venous pressures induced in the three areas. The ordinates indicate the water and electrolyte excretions plotted as a ratio obtained for each subject by dividing the lowest excretion during congestion by the average of the three control excretions.

Each dot indicates one subject, the arrow the average for the respective dots. Solid dots indicate excretions during third period of congestion, open dots during the second congestion period.

TABLE VIII

Effect of venal caval congestion on heart rate
(The data are averaged heart rates per minute per period for number of subjects indicated)

Position of balloon	Inferior vena cava above renal veins			Inferior vena cava below renal veins			Superior vena cava		
Status of balloon	During inflation			During inflation			During inflation		
Period	1	2	3	1	2	3	1	2	3
Number of subjects per period, N	11	13	10	13	10	11	11	11	10
Mean value per period, N subjects	74	77	76	75	72	75	80	80	83
Mean control value for N subjects	73	75	75	72	69	73	80	78	80
P*	0.2	0.5	0.05	0.01	0.2	0.5	—	0.1	0.06

* P—Statistical probability per period determined by the method of *critical ratio* (*t* test).

cant. With continued congestion, filtration rate increased in some subjects so that the decrease was no longer significant. After release of congestion both of these renal hemodynamic functions returned quickly to control levels.

In the arterial blood pressures (Table VII), the individual changes were more consistent, and the small falls (− 8 to − 10 mm. Hg) in both systolic pressure and pulse pressure represented significant decreases during the first 10 minutes and probably significant decreases during the remainder of the vena caval congestion. Diastolic and mean arterial pressures, heart rate (Table VIII), and electrocardiogram again remained unchanged. Prompt return to initial values occurred in all pressures upon release of the congestion.

III. *Effects of inflation of the balloon in the superior vena cava*

The effects of superior vena caval congestion differed from inferior vena caval congestion in one major respect, water and electrolyte excretions remained significantly decreased *after* release of congestion. In addition, significant renal hemodynamic changes were absent and small, but significant alterations in arterial blood pressure occurred.

During the venous congestion, water excretion decreased variably but progressively and by the third period was significantly reduced. A fall occurred in 10 of 12 subjects. During the post-deflation measurements, urine flow remained significantly reduced in the first and second periods and possibly even in the third. Sodium excretion fell in all 12 subjects during the venous congestion and the decrease was significant in all three periods. Thirty minutes after release of the congestion, sodium excretion was still significantly decreased. Potassium and chloride excretions were affected similarly.

The mean value for renal plasma flow decreased by 13 per cent during venous congestion (compared with 25 per cent and 27 per cent during congestion of the inferior vena cava) and returned to control levels following deflation of the balloon. The individual changes were variable and almost always small so that no significant change in the group effect occurred. Glomerular filtration rate did not change during or after congestion.

With regard to arterial blood pressure, there was a small (− 6 to − 7 mm. Hg) but significant decrease in pulse pressure during all three periods of congestion and probably significant decreases in mean pressure (− 2 mm. Hg) in the third inflation period and in systolic pressure (− 5 mm. Hg) in the first inflation period. Diastolic pressure remained unchanged as did the heart rate and electrocardiogram.

IV. *Relationship of electrolyte and water excretions to the increase in venous pressure*

During congestion of each of the three vena caval areas, the decreases in excretion of sodium, potassium, and water were not related quantitatively to the degree of elevation of venous pressure, up to final venous pressures of 100 mm. to 250 mm. saline (Figure 4). A chance observation suggests that the decreased water and electrolyte excretions were, nevertheless, probably associated with some rise in venous pressure and were not simply due to local alterations in vascular volume.

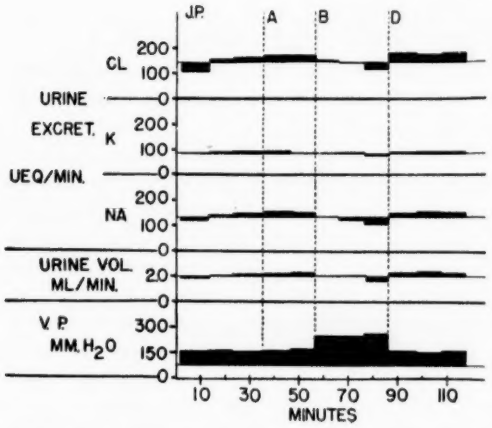

FIG. 5. WATER AND ELECTROLYTE EXCRETIONS DECREASED DURING INFLATION OF BALLOON IN THE INFERIOR VENA CAVA ABOVE THE RENAL VEINS ONLY WHEN AN INCREASE IN DISTAL VENOUS PRESSURE WAS INDUCED

At line A the balloon was inflated in the vena cava at the level of the mid-hepatic shadow. No rise in femoral venous pressure resulted. At line B the balloon was deflated, positioned at a slightly higher level in the vena cava, and reinflated. The distal venous pressure now rose and electrolyte and water excretions decreased. At line D the balloon was deflated.

For details of plotting see Figure 1.

In subject J. P. (Figure 5) a fully dilated balloon containing 9 ml. of diodrast solution was positioned in the inferior vena cava at the level of the mid-hepatic shadow. The balloon appeared easily of sufficient size to obstruct venous return and congest the vena cava. The distal venous pressure, however, did not rise but remained at 85 mm. of saline. Water and electrolyte excretions did not change. The balloon was deflated, moved upward slightly and reinflated with 8.5 ml. of diodrast solution. It now lay at the upper border of the hepatic shadow. Distal venous pressure rose to 175 mm. saline, and water and electrolyte excretions promptly fell.

V. *Relationship of electrolyte and water excretions to area congested*

Although qualitatively similar for the three caval areas, the decreases in electrolyte and water excretions were most marked during congestion of the inferior vena cava including the kidneys and less marked during inferior vena caval congestion below the kidneys and during superior vena caval congestion. Thus, mean sodium excretion decreased to 54 per cent of the control value during inferior vena caval congestion, to 67 per cent during inferior vena caval

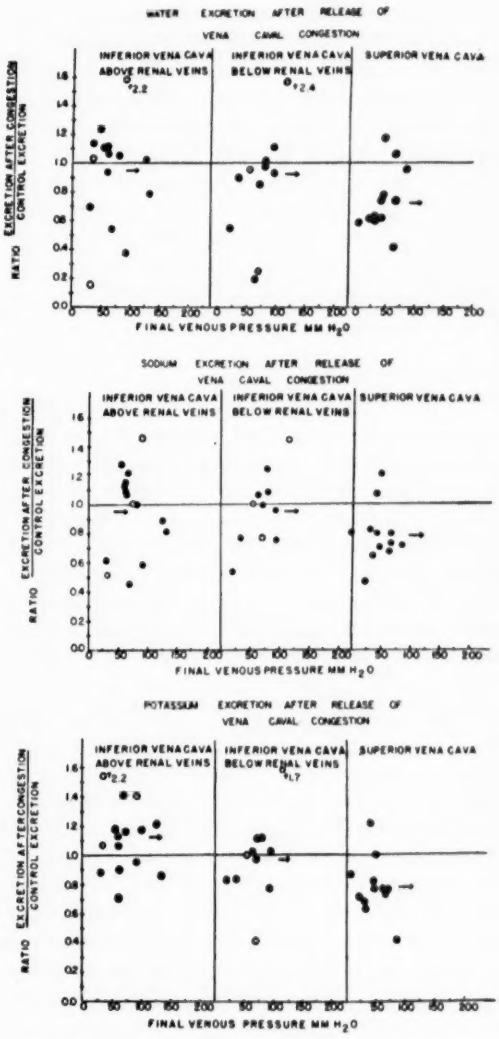
WATER EXCRETION AFTER RELEASE OF VENA CAVAL CONGESTION
INFERIOR VENA CAVA ABOVE RENAL VEINS
INFERIOR VENA CAVA BELOW RENAL VEINS
SUPERIOR VENA CAVA
RATIO EXCRETION AFTER CONGESTION / CONTROL EXCRETION
FINAL VENOUS PRESSURE MM H_2O
SODIUM EXCRETION AFTER RELEASE OF VENA CAVAL CONGESTION
INFERIOR VENA CAVA ABOVE RENAL VEINS
INFERIOR VENA CAVA BELOW RENAL VEINS
SUPERIOR VENA CAVA
RATIO EXCRETION AFTER CONGESTION / CONTROL EXCRETION
FINAL VENOUS PRESSURE MM H_2O
POTASSIUM EXCRETION AFTER RELEASE OF VENA CAVAL CONGESTION
INFERIOR VENA CAVA ABOVE RENAL VEINS
INFERIOR VENA CAVA BELOW RENAL VEINS
SUPERIOR VENA CAVA
RATIO EXCRETION AFTER CONGESTION / CONTROL EXCRETION
FINAL VENOUS PRESSURE MM H_2O

FIG. 6. URINARY WATER, SODIUM, AND POTASSIUM EXCRETIONS FOLLOWING RELEASE OF INFERIOR AND SUPERIOR VENA CAVAL CONGESTION

The abscissae indicate the levels to which the distal venous pressures returned after release of the indicated vena caval congestion.

Plotting of ordinates, dots, and arrows as in Figure 4, except that open dots indicate second post-deflation period and solid dots the third post-deflation period.

congestion below the renal veins, and to 65 per cent during superior vena caval congestion (Figure 4). During congestion of these three areas, respectively, mean potassium excretion decreased to 76, 82, and 81 per cent of control values, and mean water excretion fell to 48, 92, and 71 per cent of initial levels (Figure 4). The effect on chloride excretion was similar to that for sodium. Figure 6 indicates the striking differences in the rates of recovery of the sodium, potassium, and water excretions during the 30 minutes following release of inferior and superior vena caval congestion: essentially complete return to control levels after release of both types of inferior vena caval congestion, virtually no increase above the lowest levels of the congestion period following release of superior vena caval congestion.

DISCUSSION

No attempt was made to produce a standard degree of hydration or salting of the subjects in these observations. As a result the control levels of water and electrolyte excretions varied markedly among the subjects. Furthermore, in some individuals an undesirable degree of variation occurred in the values for the three control periods, probably due to the difficulty in obtaining reproducible urine volumes in urine collection periods of short duration at low rates of urine flow. The decision was made to accept these variations in order to gain the advantage of observing the effects of vena caval congestion at various spontaneously occurring rates of water and electrolyte excretion. In spite of the variations in the control data the changes induced by vena caval congestion were, when viewed on the whole, quite decisive; the renal excretion of electrolytes and water decreased, and in most respects this effect was similar for both inferior and superior vena caval congestion. The data fail to indicate the mechanisms responsible for these reduced excretions but do permit several interesting considerations.

The decreased excretions of both sodium and chloride were usually associated with a decreased excretion of water for, in most instances, the urinary concentrations of these two electrolytes did not change materially. However, in three subjects there was little or no change in urine flow, and decreases in urinary concentrations accounted almost wholly for the reduced excretion of both sodium and chloride. With regard to potassium, urinary concentrations usually increased but not sufficiently to compensate for the accompanying reduction in urine flow. Potassium excretion accordingly fell.

Several observations suggest that the decreased electrolyte and water excretions are not related directly to changes in renal hemodynamic functions. In most instances, renal plasma flow and glomerular filtration rate decreased at some time during both inferior and superior vena caval congestion, usually early during the congestion and concomitantly with the initial fall in electrolyte and water excretions. However, as the venous congestion was maintained, both of these renal hemodynamic functions varied considerably. Often they increased and in some subjects even reached control levels during the third period of congestion, while electrolyte and water excretions continued to decrease progressively. Particularly during superior vena caval congestion were the usual decreases in electrolyte excretions encountered without measurable changes in glomerular filtration rate or renal plasma flow. The dissociation between electrolyte excretions and renal hemodynamics was even more marked after release of the venous congestion. Both renal hemodynamic functions returned promptly to their control levels during the first post-deflation period. Electrolyte and water excretions, on the other hand, continued at their low levels or decreased further during the first post-deflation period, increased slowly thereafter and at times had not reached control values in the third period. Furthermore, the prompt return of the renal hemodynamic functions to control levels following release of venous congestion indicates that the delayed recovery of the electrolyte excretions was probably not the result of a lag in the excretion of urine trapped above the bladder.

Although the altered urinary excretions appear not to depend upon decreases in renal plasma flow or glomerular filtration rate, one cannot thereby entirely discount the significance of the observed changes in these renal functions. It is still possible that the early decreases in these functions, in some manner, initiated a sequence of events which then caused the reduced electrolyte and water excretions.

Alterations in intrinsic renal pressures (arterial, glomerular, venous, and tubular) have been

shown to decrease the excretion of urine by physical mechanisms (18–21). Such mechanisms probably do not play a major role in the decreased electrolyte and water excretions here reported. During inferior vena caval congestion including the renal veins, the mechanisms associated with altered renal venous pressure and, perhaps, altered ureteral and glomerular pressures may have been responsible, in part, for the decreased urinary excretions. However, it is difficult to consider these mechanisms operating during congestion of the superior vena cava or of the inferior vena cava below the renal veins, and it is significant that during congestion of each of the three vena caval areas essentially similar effects were produced. A small change in systemic, hence renal, arterial blood pressure was common to congestion of each of the caval areas. Again, it appears unlikely that these small reductions in arterial pressure were responsible for the reduced electrolyte and water excretions. Certainly the arterial pressure changes were much smaller than the considerable changes in renal arterial pressures which failed to influence electrolyte and water excretions in one series of animal experiments (19). In other animal studies a decided fall (20 mm. Hg or more) in mean arterial pressure was induced before glomerular filtration and urine flow decreased (22). Furthermore, in several patients, for example R. D., systemic arterial pressure rose during the venous congestion, yet electrolyte and water excretions decreased as usual.

One can conclude, therefore, that the data do not indicate a renal hemodynamic mechanism as the major factor in the decreased electrolyte and water excretions during vena caval congestion. Other renal mechanisms, secondary to primary extra-renal changes, appear to be involved.

Since the vena caval congestion was produced by pooling blood into local areas and thereby altering the distribution of a normal blood volume, hemodynamic changes in the circulatory system, other than in the kidney, could have been induced. The possible participation of such hemodynamic changes in the decreased electrolyte and water excretions merits attention, even though the necessary data on most points were not obtained in this study. Consideration will be given to the following possible changes, each of which has been advocated as capable of influencing renal functions: *a*) decreased total blood volume, a consequence of outward transudation of fluid from blood in the congested area; *b*) changes in stretch of vessel walls, including the arteries, arterioles, and capillaries, as well as the veins; *c*) altered cardiac output, with altered flow in some critical organ, or organs, and consequent changes in their function. With regard to alterations in vascular stretch and in blood flow, the effect of the changes in the non-congested areas, as well as the congested areas, must be considered.

Blood volume probably did not decrease during the vena caval congestion, at least as judged by the absence of changes in plasma protein concentration and in the hematocrit. These findings, plus a similar absence of change in blood electrolyte concentrations, also suggest that the osmotic pressure of blood was not increased. Hence the anti-diuretic response to increased osmolarity of the blood (23) need not be invoked. Fluid transudation from the capillaries probably did occur in the congested areas, but apparently hemoconcentration and a reduction in blood volume were avoided by the return of the transuded fluid into the normal-pressured venous system outside of the congested area.

If the mechanism responsible for the reduced water and electrolyte excretions derives from an altered stretch of vascular walls, the data do not indicate either the vessels involved or the nature of the altered stretch. Two possibilities present themselves. If the initiating mechanism is an increased stretching of blood vessel walls, then all parts of the systemic venous system appear to react similarly, for both superior and inferior vena caval congestion induced the decreased urinary excretions. If decreased stretch is the initiating stimulus, then common to both superior and inferior vena caval congestion might be a decreased stretch of the pulmonary vascular system, an effect considered by some observers (24) to initiate reduced urinary excretions. A choice between the two possibilities, or either one, is not possible.

The failure of the decreases in electrolyte and water excretions to be related directly to the degree of rise in venous pressure does not necessarily exclude the possibility that the decreased excretions were the result of altered vascular stretch. The level to which the venous pressure was raised was purposely limited to a range be-

tween 100 mm. and 250 mm. of saline, levels comparable to those encountered in congestive heart failure. It is conceivable that within this range all increases in venous pressure may constitute a physiological stimulus (vascular stretch) of the same order of magnitude and hence provoke the same response. The considerably higher venous pressures induced by partial ligation of a renal vein (3, 4) or by inflated cuffs about the extremities (8) may then constitute stimuli of different orders of physiological magnitude and produce varying degrees of reduction in electrolyte and water excretion.

There is the possibility that stimulation of venous walls by a foreign body (the balloon) initiated the urinary effects. Several observations indicate that this is unlikely. The balloon was never inflated to the extent that it wedged in the vena cava and pressed on its walls. In all observations the balloon moved freely in the vascular lumen with respiration and with the transmitted aortic pulse. Furthermore, decreased urinary excretions did not occur when the balloon was inflated once in a subclavian vein and once in an iliac vein where the areas congested were small. Finally, there was the observation (Figure 5) in which full inflation of the balloon failed to induce decreased electrolyte and water excretions when the distal venous pressure did not rise but produced typical reductions when readjustment of the position of the balloon caused a rise in the distal venous pressure.

The mechanism by which altered stretch of vascular walls may affect renal function remains undetermined. Presumably, a neurogenic pathway, involving the sympathetic nervous system, would mediate the stimulus from the vessel wall to the kidney. Data on this point are meager and inconclusive. In some animal experiments urine flow and the excretion of sodium and chloride have been reported to increase acutely following section of the sympathetic nerves to the kidney (25). The experiments do not indicate whether these effects are the result of altered electrolyte transport or of altered hemodynamic function. In contrast are the observations in which electrolyte and water excretion still decreased when venous congestion was produced in sympathectomized (lumbo-dorsal) men by inflating cuffs about the thighs (8).

With respect to altered blood flow as a possible mechanism responsible for the decreased urinary excretions, the data are again meager. A preliminary observation suggests that cardiac output decreases during the venous congestion induced in extremities by inflated cuffs and that the associated reductions in electrolyte and water excretions are prevented, or returned toward control levels, when cardiac output is increased by transfusions (26). However, full data concerning cardiovascular dynamics during venous congestion have not been published. Moreover, it seems unlikely that cardiac output of itself alters the renal excretion of water and electrolytes directly. A more reasonable possibility would be that altered cardiac output induces hemodynamic effects in an organ, or organs, with consequent alteration in their function, which in turn causes the decreased excretions by some renal effect. The hemodynamic alterations in one organ, the kidney, and their significance have been discussed. No data are available concerning the effect of venous congestion in altering the hemodynamics and functions of other organs.

Altered function in an organ would presumably produce renal effects by a humoral mechanism. Although the decreased electrolyte and water excretions were in general related to the period of venous congestion, implying a hemodynamic cause, the participation of a humoral component was suggested by the tendency of the decreased electrolyte and water excretions to outlast the general and renal hemodynamic effects, after venous congestion was released. This effect was particularly evident after superior vena caval congestion, suggesting thereby the participation of a humoral substance, possibly pituitary in origin, in the effects of venous congestion of the head. The participation of such a substance in the effects of inferior vena caval congestion is much less evident. In the present study, the circulation was well maintained during the venous congestion and certainly nothing approaching circulatory collapse, known to produce a release of pituitary anti-diuretic hormone (27), occurred. However, the circulatory changes which did occur were comparable to those observed during quiet standing when pituitary anti-diuresis also occurs (27). Finally, even though the observed fall in water excretion may be consistent with the known

action of the pituitary anti-diuretic hormone, this hormone is not known to produce the type of decreases in electrolyte excretion encountered in these observations.

This discussion, like this study, has raised many more questions than it has answered. Further observations are required of hemodynamic effects, both cardiac and of individual organs, or hormonal participation, pituitary and adrenal in particular, and of the neurologic component, especially the sympathetic nervous system. Finally, these studies were undertaken in order to obtain information concerning the relationship between venous congestion and edema formation in congestive heart failure. How pertinent the present data are on this point is highly conjectural. The observations dealt only with acute changes. The effects of long-continued, induced venous congestion in man remain to be determined.

SUMMARY

1. Elevated vena caval pressures, ranging from 100 mm. to 250 mm. saline, were produced for periods of 30 minutes by the inflation of a balloon in the inferior vena cava above the renal veins in 16 subjects, in the inferior vena cava below the renal veins in 13 subjects, and in the superior vena cava in 12 subjects.

2. During the venous congestion of each of the three areas essentially similar changes occurred in water and electrolyte excretions, renal hemodynamics and arterial blood pressures:

a) The urinary excretions of sodium and chloride, and less consistently of potassium and water, decreased.

b) Urinary electrolyte concentrations tended to remain unchanged and the reduced electrolyte excretions were usually due to the reduced water excretion. Occasionally water excretion decreased little or not at all, yet electrolyte concentrations fell decidedly.

c) Renal plasma flow and glomerular filtration rate usually decreased by 15 to 25 per cent at the onset of the venous congestion and concomitantly with the reduction in water and electrolyte excretions. As venous congestion was maintained, both renal hemodynamic functions improved and were returning toward control values while water and electrolyte excretions remained at their low levels or decreased further. Filtration fraction did not change.

d) Systemic arterial pressure changed little; systolic pressure and pulse pressure fell slightly (5 to 10 mm. Hg), while diastolic pressure and mean pressure remained essentially unchanged. Heart rate and the electrocardiogram remained unaltered.

e) Arterial hematocrit and plasma protein concentration did not change.

3. Following release of inferior vena caval congestion, both including and excluding the kidneys, water and electrolyte excretions, renal plasma flow and glomerular filtration rate returned to, or well toward, control levels, the renal hemodynamic functions promptly, the urinary excretions within 30 minutes.

4. Following release of superior vena caval congestion, water and electrolyte excretions returned toward control levels slowly, or not at all, and were still reduced 30 minutes after release of the congestion. Renal plasma flow and glomerular filtration rate, on the other hand, returned promptly to control values.

5. These observations indicate that acute congestion of a sizable segment of the venous system induces a decreased urinary excretion of water and electrolytes. The mechanisms responsible for this effect remain to be determined.

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THE EFFECT OF APPLICATION OF TOURNIQUETS TO THE LEGS ON CARDIAC OUTPUT AND RENAL FUNCTION IN NORMAL HUMAN SUBJECTS [1, 2]

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When blood is pooled in the lower extremities by the application of tourniquets at subdiastolic pressures to the thighs, prompt reductions occur in effective renal plasma flow (RPF), glomerular filtration rate (GFR) and renal excretion of water and electrolytes (1, 2). Similar alterations in renal function have been shown to occur in chronic congestive heart failure (3) and in head-up tilting of the normal subject (4). Since venous congestion of the lower extremities is demonstrable in all of the above situations, it has been proposed that this factor may be causally related to the functional disturbances observed in the kidney. This study represents an investigation of the pathogenesis of the changes in kidney function which occur when blood is pooled in the lower extremities by tourniquets, particularly in regard to the possible role of cardiac output.

METHODS AND PROCEDURES

All subjects were hospital patients who presented no evidence of cardiovascular or renal disease by history, physical examination and routine laboratory procedures. Studies were performed in the morning after at least 12 hours of fasting and one hour of complete bed rest. Phenobarbital, 0.1 Gm., and codeine sulfate, 64 mg., were given orally one hour prior to initiation of the procedure, and venipunctures were performed under local metycaine anesthesia. In no case was the subject unduly apprehensive.

Unless otherwise stated, hydration of the subject was accomplished by the oral administration of 200 ml. of water every 30 minutes. This was started at least one hour prior to onset of the observations and was continued until the end of the last study period. All of the patients were receiving the regular hospital diet.

RPF and GFR were determined using sodium para-aminohippurate and inulin, respectively, as described by Smith, Goldring, and Chasis (5). Clearance periods ranged from 8 to 18 minutes with an average of 11 minutes. In most experiments, blood samples for renal clearance studies were withdrawn through the cardiac catheter, the tip of which lay in either the pulmonary artery or the right atrium. In the others, they were collected from the antecubital vein. Para-aminohippurate was determined by the method of Bratton and Marshall (6) except that plasma proteins were precipitated with cadmium sulfate instead of trichloroacetic acid. Inulin was determined by the Harrison modification (7) of the

TABLE I

The effect of venous constricting leg tourniquets on cardiac index in ten normal subjects

Subject	Cardiac index†		
	Control	Tourniquets on	Tourniquets off
B. J.	2.7	2.5	2.9
E. F.	4.0	2.7	3.6
G. H.	2.4	2.5	2.7
E. D.	2.2	1.8	2.0
J. H.	3.6	3.8	3.4
K. B.	2.6	2.3	2.6
T. H.	3.6	3.0	3.7
C. G.	4.2	3.4	3.9
E. C.*	4.3	2.8	3.0
N. A.*	5.2	2.8	6.0
Mean	3.5	2.8	3.4
Per cent of control	100	80	97
S.E.	0.3	0.2	0.3
P value		<0.02	>0.5

* Tip of catheter in right atrium. In all other subjects the catheter was in the pulmonary artery.

† Control cardiac index determinations were all made at least 35 minutes before application of tourniquets. Tourniquet values were obtained 21–35 minutes after application of tourniquets. Final determinations were made 14–36 minutes after release of tourniquets.

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method of Alving, Rubin, and Miller (8). Urine sodium concentration was measured with the Perkin-Elmer Model 52A flame photometer using lithium as an internal standard. All sodium excretion values are expressed in microequivalents per minute.

Cardiac output was determined by the direct Fick method. Samples of mixed venous blood from the right atrium or pulmonary artery were obtained from an in-lying catheter introduced into the venous system through the antecubital vein (9, 10). In 8 of the 10 cardiac output studies the tip of the catheter lay in the pulmonary artery. The catheter tip was in the right atrium in the remaining two subjects. A sample of mixed venous blood was saturated with air and its oxygen content determined according to the method of Van Slyke and Neill (11). Ninty-five per cent of this value was taken as the oxygen content of arterial blood. Gasometric analyses on three-minute samples of expired air were performed by the method of Haldane (12).

In all studies, standard blood pressure cuffs were applied to the lower extremities as near to the trunk as possible. After at least two control renal clearance periods and one control cardiac output determination, the cuffs were inflated to a level just below diastolic pressure for periods ranging from 20 to 47 minutes. No subject complained of appreciable discomfort as a result of the tourniquets.

Sixteen studies were carried out in nine male and five female subjects. In the majority of the experiments an attempt was made to determine simultaneously cardiac output, RPF, GFR, urine sodium excretion, and urine volume. Due to technical difficulties this was possible in only two subjects. The following individual data were obtained: RPF in nine, GFR in eight; urine sodium excretion in nine; urine volume in nine, and cardiac output in ten. The cardiac index and renal function data are presented in Tables I and II, respectively.

Statistical analysis of the data was performed accord-

TABLE II

The effects of venous constricting leg tourniquets on effective renal plasma flow, glomerular filtration rate, sodium excretion, and urine volume in normal subjects

Patient	Control		Tourniquets on				Tourniquets off		
	Indiv.	Avg.*	1†	2	3	4	1	2	3
			Renal plasma flow (*ml./min./1.73 sq. m.*)						
J. D.	621 686 679	662	662	486	492	526	403	466	
L. L.	499 480	490	455	421			448	516	
W. D.–1	778 706	742	750	789	727	622	580	900	905
W. D.–2	663 637	650	429	524	524		457	478	
N. A.	568 548	558	529	576	350	275	548	525	440
B. J.	612 543	578	543	510	494	413	583	691	557
H. W.	551 584	568	496	559	316		594	545	
J. H.	558 473	516	553	356	388	380	301	646	
G. H.	814 739	777	518	512	588		603	674	
Mean		616	548	526	485	443	502	605	
Per cent of control		100	89	85	79	72	82	98	
S.E.		33	33	40	48	60	35	46	
P value			<0.2	<0.05	<0.001	<0.01	<0.02	<0.9	

* Average of control period values.

† Duration of the individual periods varied from 8 to 18 minutes with an average of 11 minutes. Therefore each column on the chart may be considered as approximately an 11-minute period.

TABLE II—*Continued*

Patient	Control		Tourniquets on				Tourniquets off		
	Indiv.	Avg.*	1†	2	3	4	1	2	3
			Glomerular filtration rate (*ml./min./1.73 sq. m.*)						
J. D.	154 174	164	160	133	157	154	164	164	
L. L.	87 99	93	115	117			112	128	
W. D.–1	202 185	194	245	204	234	148	208	238	
W. D.–2	221 181	205	128	173	173		147	163	
B. J.	121 122	122	110	113	118	96	127	159	114
H. W.	142 158	150	129	140	109		188	161	
J. H.	148 126	137	126	86	100	108	75	179	176
G. H.	179 150	165	120	123	156		131	144	
Mean		154	142	136	150	127	144	167	
Per cent of control		100	92	88	97	83	94	108	
S.E.		13	16	13	18	14	15	11	
P value			<0.5	<0.1	<0.3	<0.05	<0.5	<0.4	
			Urinary sodium excretion (*μEq./min.*)						
J. D.	239 235	237	215	115	74	65	65	109	
L. L.	117 125	121	125	110			87	117	
W. D.–1	274 222	248	242	221	175	158	121	181	176
W. D.–2	167 198	183	154	132	132		100	124	
N. A.	233 201	217	262	194	146	153	144	155	176
B. J.	291 260	276	222	168	163	113	144	245	219
J. H.	284 278	281	302	172	186	163	100	306	412
E. F.	413 467	440	462	341	225	165	152	187	254
E. C.	127 138	133	147	86			84	111	
Mean		237	237	171	157	136	111	171	247
Per cent of control		100	100	72	66	57	47	72	104
S.E.		32	34	26	18	16	10	23	44
P value			>0.9	<0.01	<0.01	<0.01	<0.01	<0.05	<0.5

TABLE II—*Continued*

Patient	Control		Tourniquets on				Tourniquets off		
	Indiv.	Avg.*	1†	2	3	4	1	2	3
			Urine volume (*ml./min.*)						
L. L.	11.0 10.0	10.5	8.9	5.0			1.8	3.9	
W. D.–2	5.3	5.3	4.6	1.0			0.7	0.7	
B. J.	8.6 8.3	8.5	7.3	2.6	1.0	0.9	1.3	3.6	6.5
H. W.	3.3 4.1	3.7	3.4	2.2	1.2		2.1	2.3	
E. F.	6.9 8.0	7.5	8.9	4.3	1.8	1.6	2.1	2.5	4.5
J. H.–1	9.3 7.4	8.4	5.0	1.8	1.5	1.8	0.6	1.9	2.9
J. H.–2	8.7 8.1	8.4	3.2	1.3	1.1				
G. H.	4.6 5.9	5.3	3.8	1.2	0.9		0.6	1.2	
C. G.	9.8 10.7	10.3	4.1	1.2	1.3		0.6	0.5	1.2
Mean		7.5	5.4	2.2	1.3	1.4	1.2	2.1	3.8
Per cent of control		100	72	29	17	19	16	28	51
S.E.		0.8	0.8	0.5	0.2	0.6	0.2	0.5	
P value			<0.05	<0.001	<0.001	<0.01	<0.001	<0.001	<0.1

ing to the methods described by Snedecor (13). Each tourniquet and post-tourniquet period has been compared with the corresponding control period and the group P values calculated accordingly.

RESULTS

Since in only two of the subjects were simultaneous data on all the above functions obtained, it is impossible to correlate cardiac output changes with renal function changes in individual studies. Furthermore, it is doubtful that the accuracy of the individual determinations would support such an attempt. For these reasons the results are presented collectively.

Cardiac index

The average control value for cardiac index was 3.5; the average during tourniquet periods was 2.8. In only two of the ten studies did cardiac index fail to fall following the application of tourniquets, thus leading to a statistically significant drop ($P < 0.02$). Repeat determinations were made following release of the tourniquets in all ten of these studies. In only one subject did cardiac index fail to rise following release of the tourniquets, and the average post-tourniquet value was 3.4. There was no significant difference between the pre- and post-tourniquet control values ($P > 0.5$). This finding conveys added importance to the drop during the tourniquet periods.

Renal plasma flow

During the first tourniquet period there was only a very slight fall in RPF ($P < 0.2$). However, a significant and progressive decline ($P < 0.05$, $P < 0.001$ and $P < 0.01$) occurred during the last three tourniquet periods. This depression of RPF extended into the first period following release of the tourniquets ($P < 0.02$), although there was a return toward control level. During the second post-tourniquet period RPF returned to control level ($P > 0.8$).

Glomerular filtration rate and filtration fraction

In all subjects except L. L., there was a slight fall in GFR during the periods of tourniquet application. The changes were minor, however, and did not approach the magnitude of those noted in RPF. Only during the fourth tourniquet period was the alteration of borderline statistical significance ($P < 0.05$). Following release of the tourniquets, GFR returned rapidly to control levels. These findings in the presence of the stated changes in RPF produced a marked rise in filtration fraction during the tourniquet periods.

Urinary sodium excretion

A highly significant depression of urinary sodium excretion ($P < 0.01$, $P < 0.01$, $P < 0.01$) occurred beginning during the second tourniquet period and lasting through the first post-tourniquet period ($P < 0.01$). Not until the third period following release of the tourniquets did sodium excretion return to control level.

Urine volume

The most marked and prolonged alteration observed occurred in rate of urine flow. Depression of urine volume was noted during the first tourniquet period ($P < 0.05$), and this was highly significant ($P < 0.001$, $P < 0.001$, $P < 0.01$) during the remaining tourniquet periods and through the second post-tourniquet period ($P < 0.001$, $P < 0.001$). Urine volume began to rise during the second and third post-tourniquet periods, but even so did not return to control level.

The collective data from all of the experiments are presented in Figure 1.

DISCUSSION

Effective circulating blood volume and intracardiac pressures were not systematically determined during the above studies. However, it has been shown by others (14) that as much as 890 ml. of blood may be pooled in the extremities when venous tourniquets are applied to both thighs and one arm, thus leading to a considerable decrease in effective blood volume. Pooling of blood in the lower extremities also results in significant reductions in venous and right atrial pressures (15) and in right ventricular end-diastolic and pulmonary artery pressures (16).

In the present study a slight but statistically significant fall in cardiac output occurred with the application of tourniquets and a rise followed their release. The mechanism of this change in cardiac output is not immediately apparent and could involve several possible factors. First, congestion of the lower extremities *per se* might, through neurogenic and humoral mechanisms, produce the observed changes. Secondly, the diminished effective blood volume which results from the pooling of blood in the extremities could be the responsible factor. In order to investigate these two possibilities, venous tourniquets were applied to the thighs of two subjects, and in both studies a slight fall in cardiac output occurred during the period of application. Without releasing the tourniquets 1000 ml. of physiologic saline solution were given rapidly into the antecubital vein and the cardiac output rose above control level in both subjects. Upon release of the tourniquets cardiac output rose even higher. The data from one of these experiments

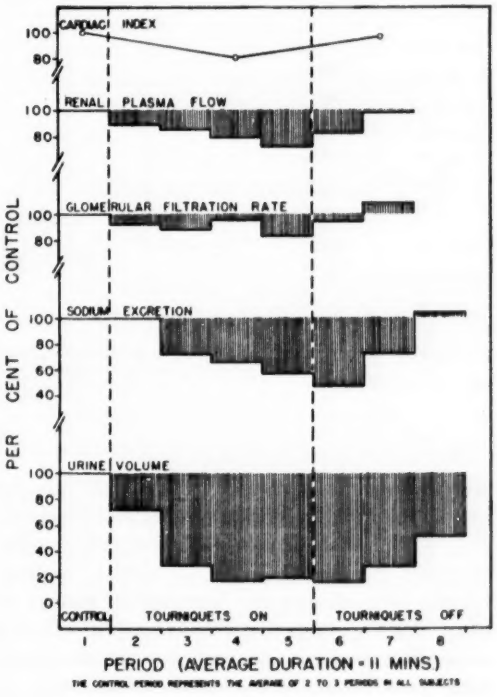

FIG. 1

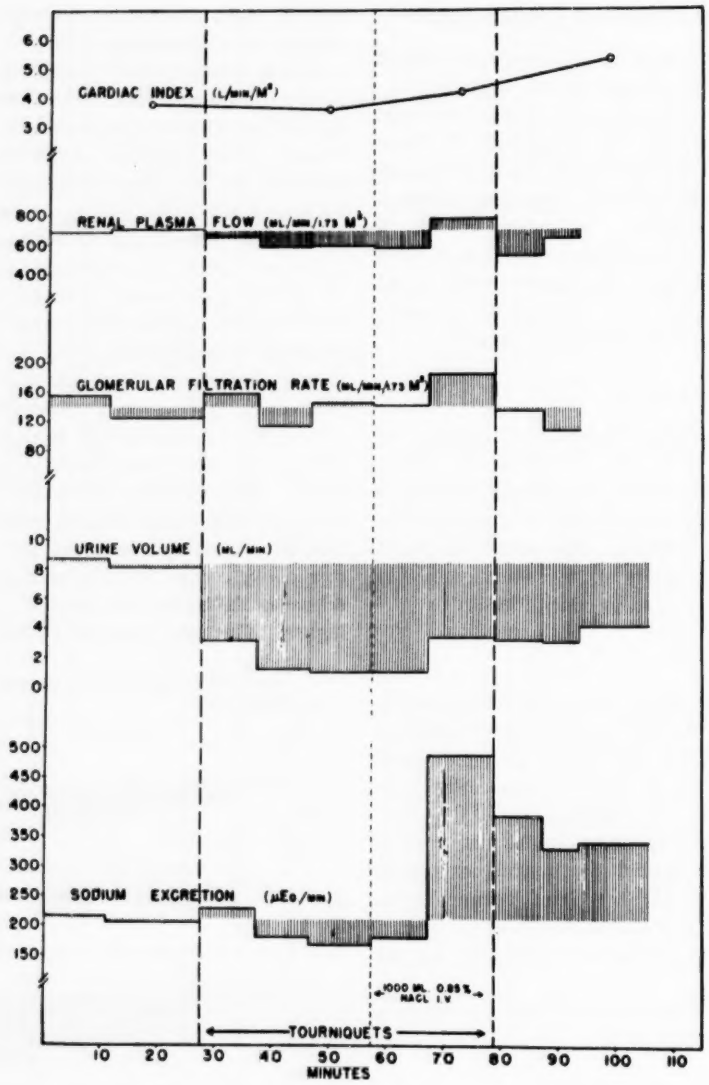

FIG. 2

are presented in Figure 2. These data indicate that by the rapid expansion of effective blood volume with physiologic saline solution, it is possible to return the cardiac output to normal or above normal even though the tourniquets remain in place. This suggests that venous congestion is not responsible for the fall in cardiac output as venous congestion would be increased, if anything, by this maneuver, and yet the cardiac output fell. A third possibility is that decreased right atrial filling pressure causes the fall in cardiac output. Since right atrial pressure was not measured in the above studies, it is impossible to comment on this factor. In the final analysis these experiments do not answer the problem regarding the fall in cardiac output, but the data suggest that diminished effective blood volume is the responsible factor.

Similarly, the renal hemodynamic effects of the

tourniquets could be produced as the result of congestion of the extremities *per se* or by the resulting decrease in effective blood volume. The experiment illustrated in Figure 2 favors the latter concept since rapid expansion of blood volume resulted in return of RPF and GFR to or toward normal. If, as postulated above, the decreased cardiac output during the tourniquet period is a result of diminished effective blood volume, then it is reasonable to state that the changes in renal hemodynamics were the direct result of diminished cardiac output. This seems likely since in many other situations a reduction in RPF has been noted in association with inadequate cardiac output. It is of interest to note that in severe congestive heart failure renal vasoconstriction produces a reduction in RPF out of proportion to the decrease in cardiac output (3). Furthermore, the renal vasoconstriction appears to be in the efferent arterioles as evidenced by the rise in filtration fraction. These same phenomena were observed in the present studies. In addition, the effects of the tourniquets upon renal hemodynamics (and upon sodium and water excretion) do not appear to be mediated through the sympathetic nervous system since they have also been observed in subjects following lumbodorsal sympathectomy (1). This has also been shown to be true of the renal hemodynamic changes of chronic congestive heart failure (17, 18).

The decrease in sodium excretion which occurred during the tourniquet periods must be secondary to increased tubular reabsorption since GFR showed only a slight fall. The rapidity with which the change in sodium excretion occurred is somewhat against an adrenocortical origin. Cerebral centers have been shown to affect sodium excretion in response to the upright posture, possibly as a result of lowered cerebral venous pressure since tourniquets about the neck inflated to subdiastolic pressures will abolish the response (19). Although these data present no definite evidence as to the cause of sodium retention during the tourniquet periods, it appears that diminished GFR plays at most a minor role.

The marked antidiuresis which occurred following application of tourniquets is similar to that observed in subjects who are tilted to the head-up position. Brun, Knudsen, and Raaschou (4) suggested that increased secretion of the neurohypophysis as a result of the diminished effective blood volume might account for the antidiuresis seen in passive tilting, and, if true, it seems likely that this mechanism may be involved in the observations here reported.

SUMMARY AND CONCLUSIONS

1. The application of venous tourniquets to the thighs results in a slight but significant fall in cardiac output; highly significant falls in effective renal plasma flow, urine flow and sodium excretion, and a fall in glomerular filtration rate of borderline significance toward the end of the tourniquet period. With release of the tourniquets all of the above measured functions return to normal. The renal changes occur at different rates; urine flow slowly, sodium excretion more rapidly, and renal plasma flow quite promptly.

2. It is suggested that the decrease in cardiac output is most likely due to diminished effective blood volume and that the diminished cardiac output in turn leads to a fall in renal plasma flow (and questionably glomerular filtration rate). The inherent errors in the available methods and the lack of methods for performing rapid successive determinations of cardiac output and renal clearances make it impossible to be certain that decrease in renal plasma flow is a result of decreased cardiac output. However, the observed changes in renal function are similar to those in other situations where cardiac output is diminished, *i.e.*, chronic congestive heart failure and shock.

3. The cause of sodium retention is not clear but seems to be due to increased tubular reabsorption.

4. The marked antidiuresis which occurs after tourniquets are applied may be due to increased activity of the neurohypophysis, occurring as the result of diminished effective blood volume.

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THE INFLUENCE OF GLUCOSE, FRUCTOSE, AND INSULIN ON THE METABOLISM OF LEUKOCYTES OF HEALTHY AND DIABETIC SUBJECTS [1, 2]

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The *in vitro* influence of glucose, fructose, and insulin on human leukocytes of healthy and diabetic subjects was measured as a part of a study of the metabolic behavior of these cells, and an attempt was made to correlate metabolic alterations in leukocytes with resistance and susceptibility to infectious diseases. Previous workers have shown that leukocytes from alloxan diabetic rabbits are able to phagocytize but not destroy bacteria (1). Dubos has shown that lactate in the presence of low oxygen tension is bactericidal for *M. tuberculosis* (2). Human leukocytes produce lactic acid in the presence of oxygen, and the quantity is not changed markedly by decreased oxygen tension (3, 4). It has also been shown that these cells produced more lactate from glucose than from fructose, and that when the two sugars were added simultaneously the lactate production was additive. The effect of these hexoses and insulin was studied on leukocytes from normal and diabetic subjects. The responses after cell injury were also determined.

METHODS

Healthy subjects and subjects with moderately severe untreated diabetes mellitus were used as donors. The obese mild diabetics were excluded from this series. The blood was collected before breakfast. The white cells were prepared for study by a method previously described (4). This consisted of the processing of the cells in a non-wetting system without exposure to excessive gravitational forces. The blood was drawn from the cubital vein into a plastic bag [5] or siliconed syringe. Dowex 50 or heparin (0.02 mg. per ml.) was used as an anticoagulant. Dextran,[6] in a final concentration of one per cent, was added to induce rouleaux formation and rapid sedimentation of the red cells. The white cells in the supernatant plasma were concentrated by centrifugation for 7.5 minutes at 50 × G. The cells were resuspended by gentle agitation in a buffered saline solution.[7] Sufficient buffer was added to obtain a cell count of 30,000 to 50,000 cells per cu. mm. In order to study the broken cells, the resuspended preparations were counted and recentrifuged at 400 × G for twenty minutes. This cell mass was ground in a mortar with alumina gel, filtered through cheese cloth, and again suspended for metabolic studies. Alumina gel was used because this system caused destruction of 90 per cent of the cells. Previous studies showed this material prepared in this manner had Krebs cycle activity (4).

The cells or brei were added to an equal volume of the buffered solution containing the hexose. The final cell counts were from 15,000 to 25,000 per cu. mm. and a total of 45,000 to 75,000 cells per vessel. The reaction mixtures were placed in 25 ml. Erlenmeyer flasks in a constant temperature water bath (37° C.) and shaken at ten oscillations a minute for four hours. Before and after incubation, lactic acid determinations were done by the method of Barker and Summerson (5). The values were expressed as micromoles of lactic acid produced by ten million white cells in an hour. The methods of Somogyi (6) and Roe as described by Umbreit,

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[2] A part of this work was presented at the Southern Society for Clinical Investigation and American Federation for Clinical Research, January 29-30, 1953, New Orleans, La.

[3] Fellow of the American Cancer Society, recommended by the Committee on Growth, National Research Council.

[4] Public Health Service Post-doctorate Research Fellow.

[5] Fenwal Blood Pack, Fenwal Laboratories, Framingham, Mass.

[6] Pyrogen free dextran, intrinsic viscosity 0.46, obtained from Pharmacia Ltd., Uppsala, Sweden.

[7] Solution prepared by diluting 10 ml. of Stock A and 7.0 ml. of Stock B in 100 ml. sterile non-pyrogenic water. One liter of Stock A contains 75.0 Gm. NaCl, 7.5 Gm. KCl, 1.0 Gm. Na_2HPO_4, 1.2 Gm. KH_2PO_4, and 0.5 Gm. K_2HPO_4. Stock B contains 10.0 Gm. Na_2HPO_4 in one liter. The hexose solutions and non-pyrogenic distilled water were supplied by Baxter Laboratories, Morton Grove, Illinois.

Burris, and Stauffer (7) were used for glucose and fructose analyses and the values were converted to the micromoles of glucose utilized by ten millon cells in one hour. In the usual experiment, the final concentration of insulin was 0.1 u per ml.[8] In a group of healthy and diabetic subjects, the response of the cells to insulin with 100 mg. per cent of glucose or fructose was measured.

RESULTS

The results of the experiments on intact and broken leukocytes from healthy subjects are graphically outlined in Figure 1. At low hexose concentration, the production of lactate was proportional to the concentration of the sugar. With glucose, there was a sharp increase with a peak at 50 to 80 mg. per cent hexose, depending on the total number of cells. As the concentration was increased above 100 mg. per cent, there was a decrease in lactate production. With fructose, the increase in lactate production was less rapid and remained linear until a concentration of 300 mg. per cent was reached. In the broken cells, the response to the two hexoses was qualitatively and quantitatively similar. When the sugars were added simultaneously, their effects were additive in the intact cell but not in the broken cell.

The utilization of glucose and fructose was measured in the intact leukocyte when the concentration of glucose was 100 mg. per cent, the fructose was 100 mg. per cent, and when the two sugars at this concentration were mixed. When the two sugars were mixed, the production of lactate was a summation of the two alone. The utilization of the hexoses, however, was markedly altered. The utilization of glucose when separate was 0.24 ± 0.09 (SE) micromole per 10 million cells per hour for glucose and when added with fructose was 0.46 ± 0.16. The fructose utilization under similar conditions was 0.16 ± 0.02 micromole per 10 million cells per hour and when glucose was added dropped to 0.04 ± 0.005.

The experiments with intact cells from normal and diabetic subjects with 100 mg. per cent of glucose and fructose, as well as the effect of insulin in the presence of glucose, are presented in Table I. The cells of the diabetic subject have a significantly lower (p 0.01) production of lactic acid with glucose and a significantly higher (p 0.01) production with fructose. This is in contrast to the normal cell. With the addition of insulin, there was a significant increase in the lactate production in the diabetic subject with no response in the control group. Data not included in the table indicated that insulin was effective in concentrations as low as 0.01 u per ml. and did not influence the lactate production in the presence of fructose. Injury or destruction of the cell membrane abolished the insulin response.

The utilization of glucose was significantly lower in the diabetic than in the control group (p 0.02). There was a significant increase (p 0.02) in utilization of glucose in the diabetic group (0.18 ± 0.04 micromole) on the addition of insulin. This brought the value for diabetic leukocytes to the normal range. Insulin did not affect the glucose utilization of the normal leukocyte at 100 mg. per cent level when 0.1 u insulin was used.

[8] Insulin obtained from Eli Lilly Co., Indianapolis, Ind.

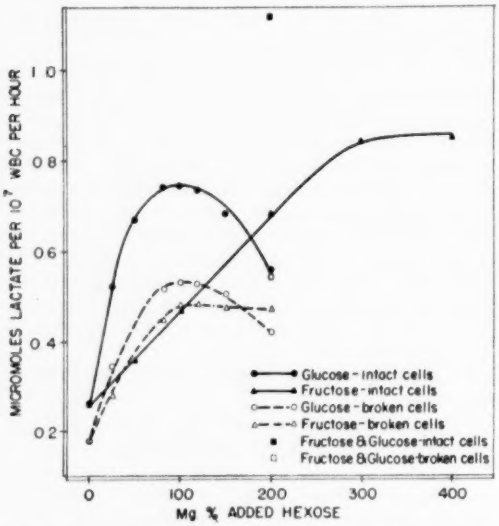

FIG. 1. THE EFFECT OF GLUCOSE AND FRUCTOSE ON THE AEROBIC PRODUCTION OF LACTIC ACID BY LEUKOCYTES FROM HEALTHY SUBJECTS

Curves represent a composite of duplicate determinations on seven subjects.

DISCUSSION

The data presented would indicate that the lactate production of human leukocytes was proportional to the hexose utilization and may be determined by the ability of the cell to transport the

TABLE I

The influence of glucose, fructose, and insulin on human leukocytes from normal and diabetic subjects (The mean and standard error of micromoles of lactate produced or glucose utilized per ten million cells per hour)

Subject	Substrate	Micromoles lactate *10 M WBC/hr.*	Change on addition of insulin	Micromoles glucose *10 M WBC/hr.*
Control 9 subjects	100 mg. % glucose	0.55 ± 0.05	+0.003 ± 0.01 (p 0.10)	0.20 ± 0.02
	100 mg. % fructose	0.38 ± 0.03		
Diabetes mellitus 22 subjects	100 mg. % glucose	0.42 ± 0.03	+0.092 ± 0.03 (p 0.02)	0.12 ± 0.03
	100 mg. % fructose	0.54 ± 0.01		

sugar across the cell membrane. The differences in response of leukocytes in the control group to glucose and fructose could be due to different mechanisms of absorption. This could be through a separate transport mechanism, different affinity for the sugars by the same mechanism, or through variations in cell permeability. Cori and Slein (8) reported a glucokinase and fructokinase in rabbit muscle. Meyerhof and Wilson (9) and Colowick (10) have described different affinities of one hexokinase for glucose and fructose. If this were the mechanism, in the present experiments at 100 mg. per cent, the ratio would be 2 to 1, a figure close to that obtained for rat muscle and brain hexokinase by the above investigators. The work of Cori on absorption of hexoses from the small intestine of rats also showed a similar ratio (11). The increased glucose utilization in the presence of fructose with a drop in fructose utilization cannot be explained. The behavior of the broken cells in these studies closely parallel the studies of Levene and Meyer (12, 13) on exudate leukocytes.

The absence of response to insulin in injured cells and the diminished response to glucose may be related. Previous work with similar preparations of leukocytes had shown that destruction of the cell integrity will also interfere with the action of hydrocortisone, a hormone which diminishes the utilization of glucose and the production of lactate by intact leukocytes (14).

Leukocytes from diabetic subjects produce less lactate in the presence of glucose. The studies of Tolstoi (15) showed no difference in glucose utilization of whole blood, but these were carried out with a different cell population, the red cell being the predominant cell. The erythrocyte and leukocyte might not have the same mechanisms of sugar transport or lactate formation. The diabetic white cell retains the ability to respond to fructose. This is comparable with the *in vivo* effects noted by Root, Stotz, and Carpenter on oral feeding (16) and more recently by Miller and his co-workers after intravenous infusion (17).

The normal leukocyte showed no response to insulin. Eadie, Macleod, and Noble (18) found that there was no response in glycolysis of whole blood of normal animals after the addition of insulin. Macleod (19) reported similar findings in sterile exudate leukocytes of normal animals. The present results indicate that the leukocyte of the diabetic subject responds to insulin by increasing lactate production. There has been a consistent discrepancy between the lactate produced and the glucose utilized. This is greater with the diabetic cells. The source of this lactate may not necessarily be through glycolysis in the complete Meyerhof-Emden System but may be through some other pathway such as that suggested by Cori (20). It may indicate the conversion of 3-phosphoglyceric aldehyde to methyl glyoxal and lactic acid as described *in vitro* by Meyerhof and Lohman (21). Work, currently in progress by McKinney (22), indicates the existence of glyoxylase in leukocytes.

The effect of the metabolic defect noted in the leukocytes from subjects with diabetes mellitus may account for the alterations in susceptibility to infection. Dubos (2) has shown that lactate in the presence of low oxygen tension has a very deleterious effect on the tubercle bacillus. In an area of inflammation, the leukocyte of the diabetic subject might be unable to utilize the available glucose to produce bactericidal quantities of lactic acid.

SUMMARY

Leukocytes from healthy individuals produce more lactic acid from glucose than from fructose,

and fructose increases the glucose utilization. This difference is abolished by grinding the cells. Leukocytes from diabetic subjects produce more lactic acid from fructose than from glucose. In the healthy subject, insulin had no measured effect on the lactate production of the cell; while in the diabetic, it produced a significant increase in lactate formation with glucose but not with fructose. The utilization of glucose was significantly increased by insulin in the diabetic group but not in the control group. Cell injury blocked the hormone effect.

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THE COMBINED EFFECT OF CORTISONE ACETATE AND DESOXYCORTICOSTERONE ACETATE IN ADRENALECTOMIZED RATS ON A LOW SODIUM DIET [1]

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In a previous report the authors have described the response of young adrenalectomized rats to desoxycorticosterone acetate (DCA) and to cortisone acetate (CA) when maintained on a diet severely restricted in sodium content (1). Daily injections of 2.5 mg. of DCA resulted in progressive weight gain and maintenance of normal blood pressure, while similar doses of CA were accompanied by rapid weight loss and arterial hypertension. With both steroids terminal serum sodium values were within normal range.

The present experiments describe the results obtained over a wide range of dosages of DCA [2] and CA,[3] given singly and in varying combinations, and were undertaken in an attempt to determine to what extent the individual action of each steroid was modified by concomitant administration of the other.

METHODS

Nine separate experiments were conducted over the period of a year and a half, employing a total of 425 female rats of the Sprague-Dawley strain. The animals were purchased at a weight of 90 to 100 Gm.[4] The rats were housed in individual cages and, after a one or two week control period, were adrenalectomized under ether anesthesia. Sodium restriction was instituted in all groups immediately postoperatively with the exception of one group of 19 control rats, in which a four-day recovery period on a high salt regimen (*i.e.*, 1.7 per cent NaCl in the diet, plus 0.9 per cent saline as drinking water) intervened between adrenalectomy and the introduction of the experimental regimen. The details of the low sodium diet have been reported previously (1). Analyses on three occasions in the past year, since the substitution of low sodium yeast [5] for the debittered form, have yielded 0.36, 0.35, and 0.40 mEq. of sodium per 100 Gm. of diet. Immediately postoperatively, sodium restriction was instituted and at the same time injections of the steroid to be tested were started, and repeated daily. The varying doses of DCA were dissolved or suspended in 0.25 ml. of sesame oil. The CA was suspended in normal saline or in the following vehicle: Tween 80, 0.4 per cent; sodium carboxymethylcellulose, 0.5 per cent; benzyl alcohol, 1.5 per cent; and sodium chloride, 0.9 per cent. The varying doses of CA were given in a total volume of 0.25 ml., except in the groups receiving 2.5 mg., in which 0.5 ml. were given. Weights were recorded daily. Blood pressures, using the microphonic manometer method described by Friedman and Freed (2) were determined prior to adrenalectomy and again four to eight days after the institution of the experimental regimen. The animals were sacrificed at the end of six to ten days by exsanguination from the incised heart under nembutal anesthesia. Serum thus collected from individual animals was pooled according to group and analyzed for sodium content.

RESULTS

The results are set forth in the three accompanying tables.

Table I: Changes in body weight. The two groups of untreated adrenalectomized rats sustained weight losses of 16 and 25 Gm. during the experimental period. In the groups receiving DCA alone, doses of as little as 0.005 mg. resulted in less weight loss than in untreated animals. With increasing amounts of DCA there

[1] This research has been supported by a grant from the U. S. Public Health Service, National Institutes of Health (H-1302) and the Albert and Mary Lasker Foundation.

[2] DCA, desoxycorticosterone acetate, was supplied through the generosity of Dr. Kenneth Thompson of Organon, Inc., Orange, N. J.

[3] CA, cortisone acetate, was made available through the kindness of Merck and Company, Rahway, New Jersey.

[4] From the Holtzman-Rolssmeyer Co., Madison, Wisconsin.

[5] Bowman Food Yeast, furnished through the courtesy of Charles Bowman & Co., New York.

obtained a straight line correlation between the log dose of this steroid and the overall weight change, until the dosage of 2.5 mg. was reached. Whether the slow rate of gain in this group of animals was the result of conditions peculiar to that one experiment or whether it constitutes a consistent finding requires additional data. In groups of rats receiving varying doses of CA alone no dosage level was encountered which prevented weight loss, and at the two highest dosage schedules employed (1.25 and 2.5 mg. daily) the rats lost weight even more rapidly than the untreated control groups. When the two steroids were given in combination, the growth-promoting effects of DCA predominated until the daily dose of CA was in excess of 0.32 mg. At the dosage level of 1.25 mg. of CA, no weight gain was seen even when large doses of DCA were given concomitantly, while at the level of 2.5 mg. the CA marked weight losses occurred despite DCA.

Table II: Blood pressure. A progressive fall in blood pressure to unobtainable levels ensued in the untreated adrenalectomized rats when subjected to the sodium restricted conditions of this experiment. In the groups given DCA alone the blood pressure was maintained at low normal to normal levels. Daily doses of 0.08 mg. and above were perhaps more effective in this regard, but even in the groups receiving only 0.005 mg. daily some blood pressure sustaining action was apparent. At no dosage level was hypertension seen in the animals receiving DCA alone. In the groups given CA, blood pressure was sustained less consistently than with DCA at the lower dosage levels. However, as the dosage was increased the blood pressure rose and in the groups receiving 2.5 mg. daily definite hypertension was evident as early as six days after the institution of the regimen. When combinations of DCA and CA were given, the hypertensive action of the

TABLE I

*Weight change of groups of adrenalectomized rats after six days of sodium restriction and daily injections of DCA and CA, singly and in combination**
(Grams)

Daily dosage of cortisone acetate (CA) in milligrams	Daily dosage of desoxycorticosterone acetate (DCA) in milligrams													
	0		0.005		0.02		0.08		0.32		1.25		2.5	
	Aver.	S.D.†	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.
0	−16	7.4	− 7	4.6	− 2	5.7	+5	4.7	+10	4.1	+17	3.2	+ 7	7.2
	−25	2.8	−13	4.6	− 7	3.5	+4	4.1	+ 9	4.4	+15	3.3		
			−15	5.7	−10	4.9	+1	7.1	+ 6	4.5	+15	3.6		
							+1	8.1			+15	4.8		
							−1	4.4			+13	2.9		
0.005	−20	7.3												
	−20	9.6												
0.02	−17	5.8					−2	6.0	+12	4.4				
	−22	6.9												
	−26	6.3												
0.08	−22	5.5					0	3.5	+12	1.8				
	−23	5.4												
	−25	11.3												
0.32	−21	8.6	−17	4.7			0	3.3	+ 7	5.9	+10	8.3		
	−21	5.2					−7	4.9						
	−22	3.5												
1.25	−25	4.1			−12	6.3	−3	2.7	− 1	5.1	− 3	2.6	− 5	4.7
	−28	7.1			−17	9.5	−4	6.5	− 1	1.4	− 5	7.2		
	−30	5.4					−4	8.2	− 4	6.0				
2.5	−32	3.2									−22	6.7	−19	19.5
	−35	3.7												

* Each value represents the average weight change of 6 to 19 individual rats. Where two or more values are recorded for one dosage schedule, each represents the result of a separate experiment.

† S.D. = $\sqrt{\Sigma \frac{(x - n)^2}{n}}$.

TABLE II

*Blood pressure readings on groups of adrenalectomized rats after four to eight days of sodium restriction and daily injections of DCA and CA, singly and in combination** (*mm. Hg*)

Daily dosage of cortisone acetate in milligrams	Daily dosage of desoxycorticosterone acetate (DCA) in milligrams											
	0		0.005		0.02		0.08		0.32		1.25		2.5	
	Aver.	S.D.†	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.	Aver.	S.D.
0	<70 ± 4.8		106 ± 13.0		119 ± 9.6 99 ± 14.2		125 ± 6.8 123 ± 10.6 113 ± 10.9		135 ± 9.3 125 ± 6.4		134 ± 1.1 133 ± 9.0 125 ± 7.5		143 ± 14.5	
0.005	98 ± 19.8													
0.02	102 ± 15.6 102 ± 21.5 <70 0						121 ± 11.1		127 ± 4.3					
0.08	107 ± 19.2 97 ± 21.8 79 ± 17.2						119 ± 3.8		123 ± 8.1					
0.32	130 ± 8.6 117 ± 19.6 111 ± 14.6		127 ± 8.0				139 ± 8.4 137 ± 7.2		133 ± 11.2		136 ± 9.0			
1.25	158 ± 11.9 151 ± 12.1 143 ± 13.7				150 ± 6.9 145 ± 7.7		155 ± 9.3 147 ± 9.0 147 ± 4.4		161 ± 9.3 154 ± 7.8 144 ± 5.9		156 ± 12.6 152 ± 13.3		157 ± 7.7	
2.5	179 ± 7.5 173 ± 10.7										155 ± 12.4		170 ± 12.6	

* Each value represents the average of blood pressure readings on a group of 6 to 10 individual rats. Where two or more values are recorded for one dosage schedule, each represents the result of a separate experiment.

† S.D. = $\sqrt{\Sigma \frac{(x - n)^2}{n}}$.

latter was not interfered with over a wide range of DCA dosage.

Table III: Terminal serum sodium values on pooled sera. In the untreated adrenalectomized rats a sharp reduction in the serum sodium was observed consistently. In the groups given DCA alone, the serum sodium concentration rose progressively as the dosage of the steroid was increased, to reach normal levels in the range of 0.08 to 0.32 mg. daily. Additional increases in dosage were without further effect upon the serum sodium. In the groups receiving CA alone no effect upon serum sodium was evident until a daily dosage of 1.25 mg. was reached, when low normal to normal values were obtained. In the hypertensive rats on 2.5 mg. daily, sodium values were, as known previously, entirely normal. In groups given a combination of the two steroids the concentration of sodium paralleled the dosage of DCA, except in the lower dosage range, where the values suggest an additive effect, although the data are too meager to permit a definitive statement.

DISCUSSION

At the outset of the currently reported experiments it seemed that the effect of desoxycorticosterone acetate (DCA) upon growth in the young adrenalectomized rat (3) might be used, along with observations upon the serum sodium values, as a tool for developing a simple assay method for DCA-like activity. The consistency of the response as seen in the above experiments proves this to be a useful technique. Before applying this assay to biological materials it was essential to know to what extent the possible presence of cortisone-like steroids, with their known suppressive effects upon growth might interfere with the characteristic DCA response. Ingle (4) had reported that no inhibition of growth was evident until daily doses of 5 mg. of cortisone acetate (CA) were given concomitantly with the

TABLE III

Terminal serum sodium concentrations in groups of adrenalectomized rats after six to ten days of sodium restriction and daily injections of DCA and CA, singly and in combination (mEq. per liter)*

Daily dosage of cortisone acetate (CA) in milligrams	Daily dosage of desoxycorticosterone acetate (DCA) in milligrams						
	0	0.005	0.02	0.08	0.32	1.25	2.5
0	122.1	123.7 123.3	131.4 125.5	135.8 135.2 135.1 131.7	140.8 140.0	141.4(5) 140.8 139.0 135.3	
0.005	119.3(2)						
0.02	125.1 119.3(5)			135.4	138.6		
0.08	124.7(4) 119.3 118.7(2)			136.4	138.6		
0.32	123.1 121.1 120.4(5)	129.6		138.4 136.4	140.5	138.4	
1.25	138.0(3) 135.5 134.2		141.4	146.5 134.0(4)	141.4 140.5	144.2(5)	
2.5	140.5						

* Each value represents the sodium concentration in the pooled sera of 6 to 8 individual rats. When less than this number were included in a pool, the figures in parentheses indicate the number sampled. Where two or more values are recorded on one dosage schedule, each represents the result of a separate experiment.

DCA. However, under the sodium restricted conditions of the present study the addition of CA in doses exceeding 0.32 mg. daily regularly inhibited the anticipated growth response to DCA. At levels of CA in excess of 1.25 mg. this steroid could be detected by the appearance of hypertension, but there may well exist a considerable range in which sufficient CA could be present to suppress growth and yet be insufficient to raise the blood pressure. Hence, this will not provide a useful assay method for DCA when CA-like materials are present unless the amount of the latter substances can be determined separately.

The data presented, on the effects of DCA and of CA singly and in combination over a wide range of dosage upon body weight, blood pressure and serum sodium concentration, serve to emphasize the wide biological differences between these two steroids. With gradually increasing doses of DCA, the adrenalectomized animals pass from a state of obvious adrenal insufficiency to one more nearly approximating normal, with improvement first in blood pressure maintenance, then increase in serum sodium to normal, and then finally normal growth. In striking contrast, with increasing doses of CA, the groups emerge from frank adrenal insufficiency with normal serum sodium concentrations but with mounting hypertension and rapidly progressive emaciation. When dosages of CA sufficient to produce the latter two manifestations are given, large doses of DCA are ineffective in preventing their appearance.

SUMMARY

Observations are presented on the effects of desoxycorticosterone acetate (DCA) and cortisone acetate (CA) given separately and in combination upon growth, blood pressure and serum sodium of adrenalectomized rats on a sodium restricted intake.

With doses ranging from 5 gamma to 1.25 mg. daily of DCA, a consistent straight line relationship was obtained between the log dose of the steroid and body growth. Some effect upon blood pressure maintenance was demonstrable with doses as low as 5 gamma, though at no level was

hypertension seen. An effect upon serum sodium concentration was first noted with doses as low as 20 gamma and above 80 gamma normal values obtained.

With doses ranging from 5 gamma to 2.5 mg. daily cortisone acetate (CA) the rats consistently lost weight and hypertension appeared at the highest dosage levels. No effect upon serum sodium concentration was evident until dosages of 1.25 mg. daily were given.

With combinations of the two steroids, the growth-promoting effects of DCA were inhibited by doses of CA in excess of 0.32 mg. daily. The presence of DCA did not interfere with the development of CA-induced hypertension.

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A COMPARISON OF RENAL BLOOD FLOW RESULTS OBTAINED IN THE INTACT ANIMAL BY THE NITROUS OXIDE (DERIVED FICK) METHOD AND BY THE PARA-AMINO-HIPPURATE (DIRECT FICK) METHOD[1]

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The nitrous oxide method as originally conceived was applied to the cerebral circulation and found to provide an accurate clinical means of determining cerebral blood flow (1). The use of an inert gas as a test substance which reaches a blood-tissue diffusion equilibrium made possible the application of a derived Fick equation and circumvented the need for direct measurement of the rate of gas uptake by the tissue. Because of this advantage, a technically modified version of the original method was recently advocated (2) as a new approach to the measurement of renal blood flow in the intact subject, human or animal, with a particular application to the anuric state, since other renal flow methods in clinical use then become totally inadequate. In that communication, a comparison of results obtained by the nitrous oxide method with those obtained by the simultaneous use of a bubble flow meter was made and the agreement found under the prevailing conditions was felt to be quite good; approximately ± 15 per cent (95 per cent limits). Following that study an opportunity has arisen during the course of other experiments to test further this method in intact dogs under more varied conditions (excluding anuria) at the same time as para-amino-hippurate (PAH) Fick renal blood flow measurements were being made. Since the PAH Fick method is recognized as the most accurate one clinically available for measuring renal blood flow, and since it too was previously found to show a good agreement with bubble flow meter measurements (3), an evaluation of the agreement obtained between PAH and nitrous oxide determinations seemed worthwhile. Such an evaluation forms the substance of the present report and appears to justify our increasing confidence in the nitrous oxide method under most conditions, providing certain important precautions are observed.

METHOD

Mongrel male dogs weighing from 19 to 32 Kg. were used. Anesthesia was produced by an intravenous injection of Pentobarbital sodium 25 to 30 mg. per Kg. Access to renal venous blood was obtained by passing a number seven venous catheter, under fluoroscopic guidance, into the left renal vein from an external jugular vein. Catheterization of the left renal vein in the dog is considered preferable to the right, because the catheter can be placed more distal to the spermatic vein on the left than to the vena cava on the right. Access to arterial blood was obtained by the introduction of a polyethylene plastic catheter into the femoral artery. Each catheter was then attached to a manifold consisting of five three-way stop-cocks. Thus multiple blood samples could be drawn quickly and consecutively. These samples were drawn into oiled, heparinized 10 ml. syringes. A catheter was placed in the bladder and urine samples were collected through it into 250 ml. volumetric flasks. This catheter was clamped off during the nitrous oxide flows.

The PAH and the nitrous oxide flows were not run in exactly simultaneous fashion. In a few experiments, the five minute nitrous oxide determination was carried out in the middle of a fifteen minute PAH clearance period while in most experiments the nitrous oxide flow was done immediately after the last PAH clearance period.

The details of the technique of the nitrous oxide flows, method of analysis of samples, and the calculation of results have been considered at length in previous reports (1, 2). In brief, a gas mixture containing 15 per cent nitrous oxide was administered *via* tracheal tube for five minutes. Multiple, simultaneous, integrated arterial and renal venous blood samples were withdrawn at intervals over the three to five minute period necessary for the nitrous oxide concentration between the blood and the renal parenchyma to reach equilibrium. The samples were

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analyzed in the Van Slyke-Neill manometric apparatus. The results were calculated from the formula

$$RBF_{N_2O} = \frac{R^{t'} \cdot S}{\int_0^{t'} (A - R)\,dt} \cdot 100,$$

where

RBF = renal blood flow/100 grams of kidney/minute,
R = renal venous concentration of N_2O,
S = partition coefficient between blood and tissue (here assumed to be unity),
A = arterial concentration of N_2O,
t′ = time of blood tissue equilibrium.

and reported in ml. per 100 Gm. of renal tissue per min. to the nearest 5 ml.

The details of the technique of PAH whole blood flow used here, the analysis of the samples, and the calculation of the results have also been discussed in previous communications (3, 4). The general outline is as follows: A constant intravenous infusion of PAH sufficient to give whole blood concentrations of 5 to 10 mg. per cent was used. Two, or more commonly, three urine collection periods of fifteen minutes each were run. Arterial and renal venous blood samples were drawn at the beginning and end of each period. The blood samples were analyzed for whole blood PAH and the urine samples for PAH using the Evelyn photoelectric colorimeter. The only important difference in the analytical procedure from that outlined in the Smith modification of the Bratton and Marshall method was the use of trichloracetic acid in a final concentration of 2.5 per cent as the protein precipitating agent rather than cadmium sulfate. The flow results were calculated to the nearest 5 ml. per min. from the Fick equation,

$$RBF_{PAH} = \frac{UV}{A - R},$$

where

RBF = total blood flow for both kidneys per minute,
UV = amount of PAH excreted in the urine per minute,
A = arterial concentration of whole blood PAH,
R = renal venous concentration of whole blood PAH.

Since the results here are expressed in terms of total flow for both kidneys whereas nitrous oxide results are expressed in terms of 100 Gm. of one kidney, it was necessary to sacrifice the animals at the end of the experiment and determine the total weight of the two kidneys so that a common denominator of flow could be reached for comparison (ml. of flow per 100 Gm. of renal tissue per min.).

The renal blood flow comparisons were made under several experimental conditions produced for the purpose of making an evaluation of renal hemodynamic and functional changes brought about by agents known or thought to play an important role in the production of anuria. When the circumstances of the experiment were appropriate to the use of both the Fick (PAH) and the gas diffusion (N_2O) method, a comparison was made. A tabulation of the existing experimental states is as follows:

1) One animal was studied during the intravenous infusion of 1:40,000 epinephrine (U.S.P.) at the rate of 0.1 ml. per min.
2) One animal was given 400 mg. per Kg. of sodium potassium tartrate intramuscularly and studied forty-eight hours later.
3) One animal was subjected to hemorrhage and studied at a time when the mean arterial pressure was 90 mm. Hg.
4) One animal was studied three and one-half hours and one studied forty-eight hours after an intravenous infusion of 7.5 ml. per Kg. of human blood of Type A and O respectively.
5) Three animals were studied two to four hours after 0.6 Gm. per Kg. of human hemoglobin had been given intravenously and two studied forty-eight hours after the same dose, and finally,
6) Three animals were studied three, four, and forty-eight hours, respectively, after 0.7 Gm. per Kg. of human globin solution had been given intravenously.

RESULTS

Thirteen flow comparisons, nitrous oxide *versus* PAH, were made in thirteen dogs under varying experimental conditions. The results of the comparison along with the existing experimental states are summarized in Table I. Both nitrous oxide flows and PAH flows are recorded in milliliters of renal blood flow per 100 grams of renal tissue per minute. The ratio of flows $\frac{RBF_{N_2O}}{RBF_{PAH}}$ is recorded in the last column. The flows as determined by PAH ranged from 90 to 325 ml. per 100 Gm. per min. so that the comparison covered flow values from the normal range down to less than one-third of normal. The ratio of flow varied from 0.82 to 1.22. The difference of this ratio from unity has been analyzed statistically. The mean ratio RBF_{N_2O}/RBF_{PAH} is 1.02. The difference of this mean from unity (+ 0.02) has a S.D. of ± 0.128, a S.E. of the mean of ± 0.036 and a t value of 0.55, indicating that at the commonly accepted level of statistical significance no systematic error is demonstrated.

DISCUSSION

A. Validity of blood flow comparison

The lack of exact simultaneity in the comparative flow determinations may be raised as an objection to the validity of the comparison. However, since the animals were kept essentially stable at a constant depth of anesthesia through the short period involved in making the two measurements there is no reason to suspect more than the usual

TABLE I

*Comparison of renal blood flow by N_2O method and PAH method (last three columns)**

Dogs	Experimental condition	RBF_{N_2O} *ml. per 100 Gm. kid./min.*	RBF_{PAH} *ml. per 100 Gm. kid./min.*	$\frac{RBF_{N_2O}}{RBF_{PAH}}$
1	During (I.V.) U.S.P. epinephrine	170	155	1.10
2	48 hrs. after injection (I.M.) of NaK tartrate	305	315	0.97
3	Hemorrhage with moderate hypotension	220	180	1.22
4	3½ hrs. after human blood infusion	260	260	1.00
5	48 hrs. after human blood infusion	300	285	1.05
6	2 hrs. after human hemoglobin infusion	160	195	0.82
7	2½ hrs. after human hemoglobin infusion	200	180	1.11
8	4 hrs. after human hemoglobin infusion	100	90	1.10
9	48 hrs. after human hemoglobin infusion	325	325	1.00
10	48 hrs. after human hemoglobin infusion	260	295	0.87
11	3 hrs. after human globin infusion	210	240	0.87
12	4 hrs. after human globin infusion	150	165	0.90
13	48 hrs. after human globin infusion	240	200	1.20
			Mean	1.02

* Mean = 1.02; S.D. = ±0.128; S.E. of the mean = ±0.036; t = 0.55.

minor variations in renal blood flow. We, therefore, do not feel that any serious error is introduced by considering the flows as simultaneous. A second objection to the validity of the comparison arises from the fact that the nitrous oxide method relates flow to a representative 100 Gm. of kidney, in this case the left, whereas the PAH method gives results in terms of total blood flow through both kidneys. One must then determine the total kidney weight in order to reduce the two flow results to a common denominator at the same time assuming that all (100 Gm.) parts of each kidney have essentially the same flow. These problems are also thought to offer no more than minor difficulty since the method of weighing the kidneys so as to include all renal blood (capillary) involved in the nitrous oxide equilibrium, but no other, can introduce only a small error; since the abnormalities being produced during the course of the studies involved both kidneys equally; since *post mortem* examination of the kidneys showed no focal vascular abnormalities; and finally, since the present experimental results with one method (PAH) recording total renal flow and another (N_2O) recording flow per unit of renal tissue involved are in good agreement.

B. *Accuracy of the nitrous oxide method*

The ratio of nitrous oxide flow to PAH flow RBF_{N_2O}/RBF_{PAH} and the standard deviation of the difference of the ratio from unity approximate the values which can be calculated from previous studies comparing the nitrous oxide and the PAH methods for renal blood flow with the bubble flow meter (*2*, 3). The experimentally determined values are 1.02 and 12.8 per cent and the calculated values 1.03 and 9.0 per cent. The accuracy of the nitrous oxide method in the intact animal then appears to have no significant systematic error and a random error of no more than ± 25 per cent (95 per cent limits). Actually it appears to have a random error little more than the ± 15 per cent (95 per cent limits) found when it was compared with the bubble flow meter, most of the remaining variability residing in the PAH determinations.

These statistics give us increased confidence in the ability of the nitrous oxide method to measure satisfactorily renal blood flow in the intact subject under most circumstances, anuria or no anuria, providing renal venous blood may be sampled. The one circumstance under which significantly large inaccuracies may arise, *i.e.*, rapid changes in renal weight, have been alluded to previously (*2*).

However, if one is to obtain consistently accurate results a rather rigid attention to detail must be observed. This derives from the fact that renal blood-parenchyma equilibrium occurs early, normally in two to three minutes. Therefore, the renal arterio-venous differences in several of the samples will usually be less than one volume per cent. All catheters, syringes, and connections to the manifolds as well as the stop-cock connections themselves must have no gas leaks, there must be no air in the systems, the timing and rate of draw-

ing of blood samples must be very accurate, the gas mixture must be administered in a constant fashion without leakage, the bladder catheter, if in place, perhaps should be clamped off, and finally the analysis of samples must be carefully done by well-trained personnel.

Since the gas diffusion technique described herein appears to give satisfactorily accurate results in the determination of renal blood flow, use of the infra-red gas analyzer and the application of isotopic techniques should make this method even more feasible for use in human or animal investigation, particularly in the anuric subject. These new advances increase the ease, rapidity, and perhaps the accuracy of analysis at the same time reducing to 25 ml. or less the total amount of blood needed for one flow determination.

CONCLUSIONS

1. Renal blood flow as measured by a gas diffusion (nitrous oxide) method was compared with that obtained by the Fick (PAH) method in thirteen intact dogs.

2. A good agreement between the two methods was obtained under the prevailing conditions. The $\frac{RBF_{N_2O}}{RBF_{PAH}}$ has a mean of 1.02 and the difference of this mean from unity has a S. D. of $\pm$ 0.128, a S. E. of the mean of $\pm$ 0.036, and a t value of 0.55.

3. The results indicate that the gas diffusion (nitrous oxide) method should provide a satisfactory clinical measure of renal blood flow under most conditions, provided renal venous blood may be sampled and the details of the method are carefully observed.

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THE COMPETITION BETWEEN P-AMINO-HIPPURIC ACID (PAH) AND DIODRAST FOR RENAL EXCRETION AND EXTRACTION IN MAN [1]

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The competition between two substances for tubular excretion has been demonstrated repeatedly with orthodox clearance techniques. Smith, Goldring, and Chasis (1) found that raising the plasma concentration of diodrast depressed the excretion of phenol red. They concluded that both substances were excreted by the same mechanism. Similarly, it has been reported that intravenously administered diodrast (2) or PAH (3) retard the excretion of penicillin, that hippuric acid depresses the tubular excretion of glucuronic acid derivatives in the chicken (4), and that the amide of methyl-nicotinic acid depresses the tubular excretion of guanidine (5). Recently, it has been demonstrated that PAH in sufficient concentrations excludes phenol red from the cells or lumen of fish tubules suspended in appropriate salt solutions (6).

Smith, Finkelstein, Aliminosa, Crawford and Graber (7) found that diodrast and PAH given simultaneously had approximately the same renal clearance in man when the plasma concentration of both was low. They concluded that the two substances were excreted by the same mechanism. In none of these previous studies has the simultaneous renal extraction of the examined substances been investigated by analysis of renal vein blood. In fact, although it has been reported that renal extraction of diodrast is lower than that of PAH (8), there have been no published reports of simultaneous measurements of extraction of the two substances.

In the present study, the renal percentage extraction (E) and the tubular excretion (T) of diodrast and PAH in man were simultaneously compared in experiments during which the plasma concentration of one or both was varied over wide limits.

By "renal extraction," E, is meant the percentage of a solute removed from the plasma during the passage of blood through the kidneys. Thus,

$$E = \frac{A_c - V_c}{A_c} \cdot 100,$$

where A_c is the concentration of a solute in the arterial plasma and V_c is the concentration of the same solute in the renal venous plasma.

By the tubular excretion, T, is meant the difference between the total amount of a solute excreted per minute in the urine and the amount of this solute filtered through glomeruli. The filtered quantity is calculated as the product of the inulin clearance and the filterable fraction of the plasma diodrast or PAH, which generally are considered to be 73 and 83 per cent of the total plasma concentrations, respectively.

MATERIAL AND METHODS

Seventeen healthy young volunteers—medical students and nurses—were studied in the morning in the postabsorptive state. In fifteen of the experiments, a Goodale catheter was placed in the right renal vein under fluoroscopic control. An indwelling needle was placed in a brachial artery and a catheter placed in the bladder. After each clearance period the bladder was emptied and rinsed twice with distilled water and subsequently with air. At the beginning and end of each clearance period, blood samples were simultaneously collected from the brachial artery and the renal vein, transferred to heparinized tubes, and centrifuged within 5 minutes.

In experiments 1 and 2 (Table I, Figure 1), by first giving an intramuscular injection of PAH, a low and comparatively steady plasma concentration was achieved (9). After one or two control periods, by giving an intramuscular injection of diodrast,[2] a low and fairly

[1] The investigation was aided by grants from the Swedish Medical Research Council and the foundation "Thérèse och Johan Anderssons Minne."

[2] We are indebted to A. B. Astra Co., Södertälje, Sweden, for generously supplying us with 35 per cent Umbradil (diodrast).

TABLE I

Results of renal vein catheterization experiments 1 to 4

Exp. no.	Subject	Minutes	In‡	PAH A_e‡	PAH E‡	PAH Cl‡	PAH RPF‡	PAH T‡	PAH $\frac{10T}{A_e}$‡	Diodrast A_e	Diodrast E	Diodrast Cl	Diodrast RPF	Diodrast T	Diodrast $\frac{10T}{A_e}$	$\frac{Cl_D}{Cl_{PAH}}$	$\frac{E_D}{E_{PAH}}$	
1*	Man,	13	106	60	92	592	644	27	4,5									
	26 y.,	27	120	67	91	590	649	33	4,9									Diodr. intramusc.
	b.s.a.	15	116	72	91	616	671	37	5,2	40	72	568	789	27	6,9	0,92	0,78	
	2,01	26	109	86	63	456	724	31	3,6									Start diodr. infus.
		14	101	80	61	536	880	36	4,5	544	59	508	861	221	4,1	0,95	0,97	
2*	Woman,	32	133	61	87	806	926	42	6,9									Diodr. intramusc.
	26 y.,	14	133	52	85	746	877	33	6,2	87	81	673	832	49	5,5	0,90	0,95	
	b.s.a.	29	164	51	58	715	1,233	29	5,7									Start diodr. infus.
	1,73	15	190	46	61	792	1,298	29	6,3	418	54	687	1,270	229	5,5	0,87	0,88	
		16	142	38	62	720	1,161	23	6,0	323	57	665	1,166	185	5,7	0,92	0,92	
3†	Man,	24	116							189	83	726	874	25	1,3			
	21 y.,	31	142							110	89	945	1,060	29	2,7			PAH intramusc.
	b.s.a.	18	122	76	93	652	700	41	5,4	83	86	772	897	27	3,2	1,18	0,92	
	1,92	29	147							79	70	557	796	18	2,3			Start PAH infus.
		13	137	1,010	61	505	827	392	3,9	71	67	463	691	14	2,0	0,92	1,10	
4†	Man,	17	134							141	85	722	848	88	6,2			
	24 y.,	26	131							124	86	583	678	60	4,9			PAH intramusc.
	b.s.a.	12	124	150	91	604	654	74	5,0	80	81	669	827	46	5,7	1,11	0,89	
	1,83	21	131							72	76	669	879	41	5,7			Start PAH infus.
		11	131	1,133	56	425	759	353	3,1	66	53	645	1,218	36	5,5	1,52	0,95	
		12	130	1,256	55	396	721	357	2,8	59	48	526	1,095	26	4,3	1,33	0,87	Illness

* In experiment 1–2, a low arterial plasma concentration of PAH was maintained and that of diodrast was increased to high levels.

† In experiments 3–4, a low diodrast concentration was maintained, and the PAH concentration increased.

‡ In = inulin clearance,
A_e = arterial plasma concentration in μ-mol./L.,
E = apparent renal percentage extraction,
Cl = renal clearance in ml./min.,
$RPF = \frac{\text{clearance}}{E} 100$,
T = calculated tubular excretion in μ-mol./min.,
y = years,
b.s.a. = body surface area in m^2.

steady plasma concentration of this substance was also attained (10). When the simultaneous excretion of the two substances had been observed for a sufficient time at these low plasma concentrations, the diodrast concentration was increased by giving a constant intravenous infusion until one or two more periods had been concluded.

In experiments 3 and 4 (Table I, Figure 1), the experimental design was the same but the order in which the substances were given was reversed, diodrast being given first and at low concentrations only.

In the experiments 5 to 9 (Table II), both substances were given simultaneously in equimolecular quantities. In experiment 5 (Figure 2), intramuscular injections were supplemented by constant, sustained intravenous infusions of both. At first, only a comparatively high concentration was effected, followed by a very high one of both substances. In experiments 6 to 9 (Table II), PAH and diodrast were given in equimolecular amounts by intravenous infusion only. Thus, initially, the concentrations were comparatively high and subsequently very high. In experiments 6 and 7, the renal vein was not catheterized.

In eight other experiments (Table III), a comparison was made between the simultaneous renal extractions, E, and clearances, Cl, of diodrast and PAH at low plasma concentrations only, which were sustained by simultaneous intramuscular injections.

In five experiments, the influence of the time interval between sampling and centrifugation was studied by a method similar to that described by Phillips, Dole, Hamilton, Emerson, Archibald, and Van Slyke (11). A large renal venous blood sample was drawn rapidly in subjects given both PAH and diodrast. An aliquot of each sample was centrifuged within 40 to 80 seconds and others after longer intervals up to 20 min. By using an angle centrifuge with accelerations up to 8,000 r.p.m., the blood was separated in a few seconds so that the time of centrifugation was negligible. Then, the plasma was analyzed for diodrast and PAH by the usual method, slightly modified for the small concentrations.

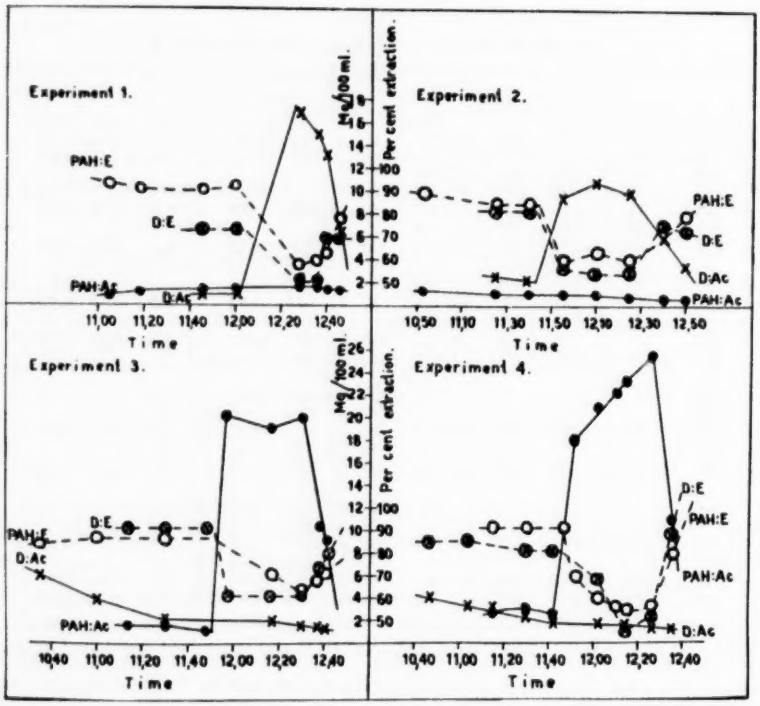

FIG. 1. RESULTS OF RENAL VEIN CATHETERIZATION-EXPERIMENTS 1-4

● = arterial plasma PAH concentration (PAH: Ac); ○ = apparent percentage PAH extraction (PAH: E); × = arterial plasma diodrast concentration (D: Ac); ⊗ = apparent percentage diodrast extraction (D: E).

The details of the methods used, abbreviations and calculations, are described in previous publications from this laboratory (12, 13, 14).

RESULTS

Table I contains the interpolated concentration in arterial blood plasma (A_c), the interpolated renal extraction (E), the renal clearance (Cl), the estimated renal plasma flow ("RPF") and the tubular excretion (T) for both PAH and diodrast for the experiments, in which the concentration of only one substance was increased. In Figure 1, the corresponding actual extraction values and arterial plasma concentrations are presented. It is evident that when the concentration of both was low, the extraction of diodrast was slightly lower than that of PAH. When the concentration of either test substances was increased, the extraction of both decreased, although the arterial content of the other was actually lower than during the control period.

In Table II and Figure 2, the results obtained during administration of high, equimolecular concentrations of PAH and diodrast are presented. When the concentrations were increased, there generally was a parallel fall in the extraction of both substances. When calculated on the usual basis of a filterability of 83 per cent for PAH and 73 per cent for diodrast, the tubular excretion, T, did not remain constant. The ratio T/A_c for PAH and diodrast is included as a comparative index of affinity for tubular excretion in the mutual competition.

In Table III are summarized the results of the first simultaneous and complete period from each low concentration experiment. The ratio of the PAH clearance to the diodrast clearance varied considerably in some of the experiments, but the

TABLE II*

Results of experiments 5 to 9 with high equimolecular plasma concentration of PAH and diodrast

Exp. no.	Subject	Minutes	In.	PAH A_e	PAH E	PAH Cl	PAH RPF	PAH T	PAH $\frac{10T}{A_e}$	Diodrast A_e	Diodrast E	Diodrast Cl	Diodrast RPF	Diodrast T	Diodrast $\frac{10T}{A_e}$	$\frac{E_D}{E_{PAH}}$	$\frac{T_D}{T_{PAH}}$
5†	Woman,	17	102	74	92	450	490	27	3,67	104	88	546	623	49	4,71	0,96	1,79
	26 y.,	22	84	Steep concentr. increase													
	b.s.a.	14	97	516	43	260	605	93	1,80	623	37	238	644	98	1,57	0,86	1,05
	1,69	21	92	697	40	236	591	119	1,71	799	40	239	597	141	1,76	1,00	1,18
		12	91	Steep concentr. increase													
		9	80	3,200	24	94	391	78	0,24	3,030	19	90	473	95	0,31	0,79	1,21
		12	83	2,530	23	112	448	103	0,41	2,360	20	119	595	137	0,58	0,80	1,33
6	Man,	19	170	896		213		66	0,74	878		272		131	1,49		2,01
	21 y.,	15	144	950		174		50	0,53	930		212		105	1,13		2,13
	b.s.a.	21	142	Steep concentr. increase													
	1,75	20	130	1,327		181		98	0,74	1,338		177		109	0,82		1,11
		22	155	1,427		141		27	0,17	1,453		153		72	0,49		2,88
7	Man,	21	113	797		259		131	1,65	854		268		158	1,80		1,21
	24 y.,	25	105	891		219		118	1,33	994		220		147	1,48		1,25
	b.s.a.	34	105	Steep concentr. increase													
	2,07	22	101	1,475		168		121	0,82	1,490		185		164	1,10		1,36
		16	94	1,835		134		103	0,56	1,865		148		149	0,80		1,43
8†	Man,	14	162	560	37	394	1,072	146	2,61	570	53	423	824	173	3,04	1,43	1,18
	20 y.,	12	158	680	41	327	802	134	1,97	610	48	403	841	177	2,90	1,17	1,32
	b.s.a.	13	141	770	44	281	642	128	1,67	680	46	350	763	167	2,46	1,04	1,30
	1,95	35	142	1,080	36	244	680	137	1,27	1,000	34	269	796	166	1,66	0,95	1,21
		11	186	1,410	27	249	934	134	0,95	1,280	29	277	956	181	1,41	1,07	1,35
		15	197	1,510	29	201	683	56	0,37	1,350	31	235	757	122	0,91	1,07	2,18
		10	182	1,640	27	211	776	87	0,58	1,460	29	241	840	160	1,10	1,07	1,84
9†	Man,	18	156	700	43	235	547	74	1,06	680	42	245	587	89	1,31	0,98	1,20
	24 y.,	16	133	820	36	216	596	86	1,05	840	42	231	547	112	1,34	1,17	1,30
	b.s.a.	15	115	900	29	220	716	112	1,24	950	40	223	579	132	1,39	1,38	1,18
	1,98	44	86	1,290	23	160	696	113	0,88	1,300	26	160	621	127	0,98	1,13	1,12
		24	108	1,950	18	138	760	95	0,49	1,970	19	147	779	133	0,68	1,06	1,41
		14	102	2,180	18	125	705	88	0,40	2,230	20	127	636	118	0,53	1,11	1,34
		13	88	2,320	17	99	583	60	0,26	2,380	19	99	517	83	0,35	1,12	1,38

* Abbreviations as in Table I.
† In 5, 8, 9, the renal vein was catheterized.

TABLE III

Comparison between the simultaneous renal clearance (Cl) and renal extraction (E) of PAH and diodrast at low plasma concentrations of both substances

Exp. no.	Sex	Age *year*	PAH Cl	PAH RPF	PAH E	Diodrast Cl	Diodrast RPF	Diodrast E	$\frac{Cl_D}{Cl_{PAH}}$	$\frac{E_D}{E_{PAH}}$
1	M	26	529	577	92	488	679	72	0,92	0,78
2	F	26	746	877	85	673	832	81	0,90	0,95
3	M	21	587	630	93	695	807	86	1,18	0,92
4	M	26	571	627	91	632	780	81	1,11	0,89
5	F	26	460	501	92	559	637	88	1,21	0,96
10	M	25	—	—	89	585	720	81	—	0,91
11	M	23	598	673	89	658	794	83	1,10	0,93
12	M	25	440	490	90	410	470	87	0,93	0,97
13	M	23	618	672	92	939	1,064	87	1,52	0,95
14	M	23	492	557	89	525	627	84	1,07	0,94
15	F	21	467	511	91	—	—	86	—	0,95
16	M	22	544	594	91	525	610	86	1,04	0,95
17	M	25	506	566	90	482	570	85	1,05	0,94
Mean			547	606	90,3	598	716	83,6	1,09	0,926
σ			85,2	105,2	2,06	138,0	154,2	4,20	0,175	0,0505
$\frac{100\sigma}{M}$			15,6	17,4	2,3	23,1	21,5	5,0	16,0	5,5

proportionality between PAH extraction and diodrast extraction was much more stable. As previously reported, the renal extraction of PAH was somewhat higher than that of diodrast, the difference between the means being highly significant ($P = 0.001$) by Student's "t" test. Statistically, the difference between the means of the clearance values was not significant. As a consequence of the lower renal diodrast extraction and the higher diodrast clearance, the calculated figures for the renal plasma flow (RPF), calculated from the diodrast data, were higher than the corresponding figures, based on PAH.

The analysis of PAH and diodrast in the renal venous samples, centrifuged after different time intervals, demonstrated that the shift of diodrast and PAH from erythrocytes to plasma after the first 40 seconds following sampling could influence the calculated extractions and RPF's only slightly (Figure 3). However, the shift during the first 40 seconds, which we were unable to study, may have been considerable.

DISCUSSION

The calculation of the tubular excretion, T, at high plasma concentrations of diodrast or PAH is subject to error from several sources. Because T represents the small difference between the large amounts filtered and the larger total urinary excretion, analytical errors are of great significance. Variations in the percentage of the plasma diodrast or PAH filterable through the glomerular membranes are probably of even greater significance. In experiments on ultrafiltration of fresh human plasma containing high concentrations of diodrast and PAH, we have obtained filterability factors which vary from 1 to 5 per cent in both directions from the classical figures of 73 per cent and 83 per cent. However, because in some experiments the filterable fraction slowly decreased, it may be that the duration of ultrafiltration is of some influence, since filtration in the capsula of Lavietes or in the centrifuged cellophane bag of Rehberg (15) took 5 to 10 hours. Moreover, filterability varied with the age of the sample. In some subjects, the changes did not exceed 1 to 2 per cent, but occasionally, after 24 hours, we found 2 to 6 per cent decreases in the ultrafilterability, and, at times, after 48 hours, decreases as great as 10 per cent. However, in fresh plasma, the proportion between the ultrafilterability of diodrast and of PAH remained reasonably steady.

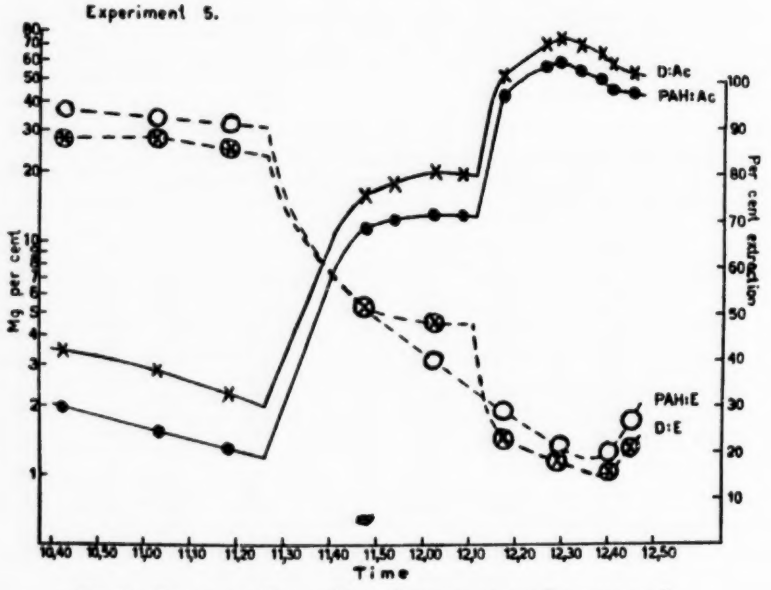

FIG. 2. RESULTS OF RENAL VEIN CATHETERIZATION-EXPERIMENT 5
Symbols and abbreviations as in Figure 1.

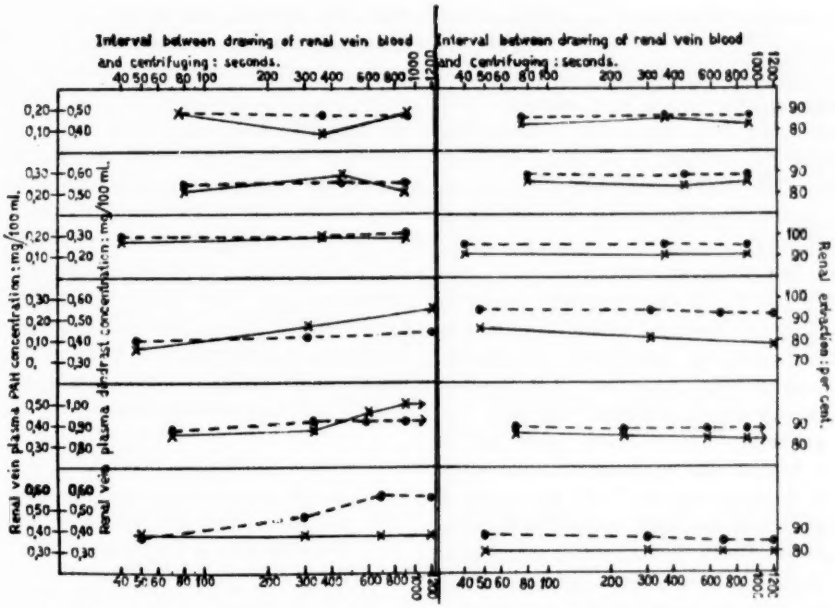
Interval between drawing of renal vein blood and centrifuging: seconds.
40 50 60 80 100 200 300 400 600 800 1000 1200
Renal vein plasma PAH concentration: mg/100 ml.
Renal vein plasma diodrast concentration: mg/100 ml.
Renal extraction: per cent.

FIG. 3. RENAL VENOUS PLASMA CONCENTRATIONS (LEFT-HALF OF CHART) AND RENAL EXTRACTIONS (RIGHT-HALF OF CHART) FOR DIODRAST (×) AND PAH (●) BLOOD CENTRIFUGED 50 TO 1,200 SECONDS AFTER COLLECTION

In the experiments recorded in the tables, the ultrafiltration was performed on 1 to 2 day old samples. Hence, we have preferred to calculate T values, with reservations, on the basis of the usual ultrafilterabilities of 73 per cent and 83 per cent.

When calculated in terms of molecules, the T-values for diodrast and PAH were essentially the same when plasma concentrations were below the saturation limit; *i.e.*, the proportion between the tubular excretion and the arterial plasma concentration, T/A_c, was of the same order of magnitude for both substances (Table I). However, at plasma concentrations above the saturation limit, the tubular excretion of diodrast exceeded that of PAH; *i.e.*, its T/A_c ratio was higher even if it were assumed that both substances were completely filterable.

The data in Table III demonstrate that at low plasma concentrations the renal plasma flow, calculated on the basis of diodrast, was significantly higher than the corresponding PAH value, although there was no significant difference between the corresponding clearances.

At low plasma concentrations, the ratio of diodrast extraction to PAH extraction was slightly below 1 and remained remarkably steady, whereas the corresponding clearance ratio was above 1 but had a high standard variation. When the plasma concentration of both substances was very high, the percentage extractions were nearly identical, with a few exceptions (Table II). If the arterial plasma concentration of one of the substances was maintained at a constant low level when the concentration of the other was suddenly increased, there resulted simultaneous and equivalent depression of both E_{PAH} and E_D (Table I).

The accumulation of diodrast in the renal tubular cells, found by Engstrom and Josephson (16), could not have induced the higher tubular excretion and renal venous concentration of diodrast. As shown with PAH by Josephson, Bucht, Ek and Werkö (13), such accumulation can influence the tubular excretion and the renal vein plasma concentration only when the plasma concentration is rapidly increased or decreased. The differences found here remained fairly steady over a long period and represented much larger

amounts of diodrast than could be explained by this process.

White, Findley, and Edwards (17), and Corcoran, Smith, and Page (18) have suggested that diodrast may shift from the erythrocytes to the plasma during the passage of the blood through the kidneys. This shift may explain the observed differences in the renal percentage extractions of diodrast and PAH.

The fact that these substances mutually depressed each other's tubular excretion indicates that either they are excreted by the same transferring mechanism, as postulated by Smith, Finkelstein, Aliminosa, Crawford, and Graber (7), or that the energy required for their excretion is provided by a common source. The fact that at higher plasma concentrations more molecules of diodrast than of PAH are excreted should result in a higher renal extraction for diodrast than for PAH under these conditions. However, the anticipated difference may be negated by the shift of diodrast from the erythrocytes to the plasma. At low plasma concentrations, when the two substances are almost equally excreted, the shift of diodrast may increase the renal vein plasma concentration, and thereby produce the apparent, lower renal extraction of diodrast with the consequent, apparent, higher renal plasma flow. The suggested shift must be so rapid that it cannot be prevented by almost immediate centrifugation of the renal venous blood. At high plasma concentrations, the percentage of plasma diodrast excreted by the tubules is small. Consequently, the plasma/erythrocytes concentration ratio is only slightly changed and the influence of the shift not measurable.

SUMMARY

Injections of PAH and diodrast were given simultaneously to 17 healthy human subjects. In 15 of these, renal venous blood for repeated analyses was obtained by venous catheterization.

When the plasma concentrations of PAH and diodrast were low, the proportions between both their respective percentage renal extractions from the plasma and their respective tubular excretions were remarkably steady.

When the plasma concentration of one of the substances was increased, the percentage renal extraction of both was equally depressed. With increasing plasma concentration, the tubular excretion of diodrast increases relative to the PAH excretion. It is concluded that the two substances are probably excreted by the same mechanism, which, at high plasma concentrations, can transport more diodrast molecules than PAH.

It is suggested that a shift of diodrast from the erythrocytes during the passage through the kidney may increase the renal venous plasma concentration. At high plasma diodrast concentrations, the influence of this shift is not measurable, but at low concentrations it leads to a lower, apparent value of renal percentage extraction for diodrast as compared to PAH.

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THE EFFECT OF MECHANICAL EXSUFFLATION ON RESPIRATORY GAS EXCHANGE IN CHRONIC PULMONARY EMPHYSEMA

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INTRODUCTION

An elevated arterial carbon dioxide tension is not infrequently found in patients with pulmonary emphysema. Donald and Christie (1) and Wilson, Borden, Ebert, and Wells (2) have shown that such patients are unable to lower their arterial pCO_2 significantly by voluntary hyperventilation. This mechanical defect in the control of blood carbon dioxide tension makes these patients particularly susceptible to the development of respiratory acidosis. An associated event is a depression of the ventilatory response to increases of carbon dioxide tension so that anoxic stimulation of the carotid and aortic bodies becomes the prime stimulus for breathing (3). When oxygen therapy is instituted in an attempt to alleviate the anoxia, the obviously distressed cyanotic patient may become almost apneic, drowsy, and even comatose. This poses a difficult therapeutic problem.

The immediate object of the therapy in such patients is to provide an adequate lung ventilation which will overcome the anoxia and increase the elimination of carbon dioxide. Often some mechanical aid to respiration is urgently required. Electrophrenic respiration (4, 5) and mechanical respirators using intermittent positive pressure breathing applied to the upper airway (6) or as applied in the conventional tank respirator (7, 8) have been recommended as an adjunct to therapy in respiratory acidosis. As the exsufflator attachment to the tank respirator has been shown to effectively reverse disturbed alveolar gases resulting from obstructed breathing (9) it was of interest to determine its effect in pulmonary emphysema.

The purpose of this paper is to present the effect of the mechanical exsufflator on respiratory gas exchange in chronic pulmonary emphysema, to compare its effect to that of the conventional tank respirator, and to describe their combined use in the treatment of a severely ill emphysematous patient who developed respiratory acidosis.

METHODS

Mechanical exsufflation, devised as a means of eliminating bronchial secretions in patients with an ineffective cough (10, 11) is accomplished in a conventional tank respirator by producing a negative intratank pressure of 40 mm. Hg for inflation of the lungs, and, by means of a swiftly opening butterfly valve, returning the intratank pressure to atmospheric in 0.06 second. Expiration therefore, in contrast to that in other forms of intermittent pressure breathing, is not impeded except for the initial 0.06 second. Expiratory volume flow rates measuring 60 per cent of the rates obtained during maximally vigorous coughs in normal subjects have been attained by this procedure (12).

In the present study the exsufflator was cycled 9 times a minute. The inspiratory pressure was built up over a 2 second period, thus allowing 4.6 seconds for expiration. The observations on the effect of the conventional tank respirator were made using an Emerson respirator cycled 17 times a minute with a pressure range of -20 to $+8$ cm. water, the time of inspiration and expiration being approximately equal. Each patient was instructed to 'breathe with' the apparatus being used.

Studies were made on 13 cases of chronic pulmonary emphysema in whom retention of carbon dioxide due to impaired pulmonary ventilation was suspected. In order to obtain a resting level all reclined in a conventional tank respirator for 30 minutes before any experiment was begun. The exsufflator was attached to the respirator so that either apparatus could be used and followed immediately by the other.

In order to determine the effect of these procedures and of the administration of oxygen, pulmonary ventilation was measured on a Benedict-Roth respirometer. Arterial blood samples were drawn by means of an indwelling Cournand needle and the Van Slyke-Neill technique was used for the determination of their oxygen content and capacity and carbon dioxide content (13). The arterial pH was determined by glass electrode at 37° C. without exposure to air on a Beckman pH meter and the carbon dioxide tension was derived by the Henderson-Hasselbach equation.

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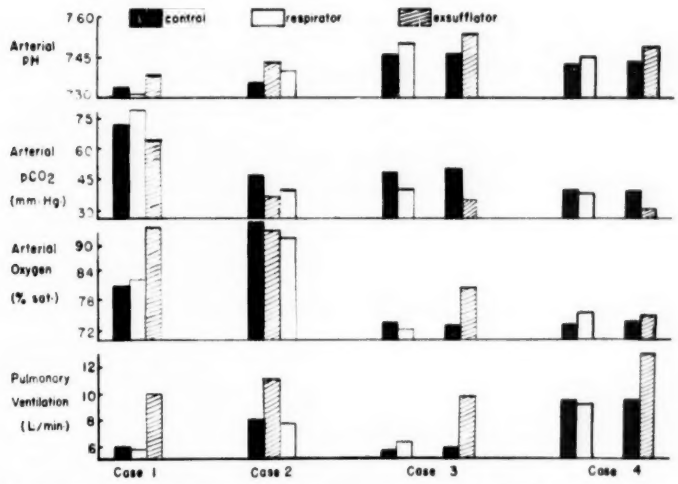

A comparison of the physiological effects of the respirator and exsufflator in emphysema.

FIG. 1

RESULTS

In four patients with emphysema the effect of the exsufflator was compared to that of the conventional tank respirator. In Case 1, 30 minutes of exsufflation were followed immediately by 60 minutes of respirator treatment. This procedure was reversed in Case 2 and broken into two parts in Cases 3 and 4, the patients receiving exsufflation for 30 minutes one day and respirator treatment for 60 minutes the next.

The effect of these two procedures on minute volume, arterial pH, carbon dioxide tension, and oxygen saturation is shown in Figure 1. The exsufflator resulted in an appreciable rise in pH, a rise in pH, a rise in oxygen saturation averaging 4.6 per cent and a fall in carbon dioxide tension averaging 10 mm. Hg while the respirator produced only a slight effect on these measurements. It is seen that the minute ventilation was practically unchanged from the resting control level during respirator therapy while the exsufflator increased the average minute volume by 4 liters per minute or by 55 per cent. The tidal volume, unchanged by the respirator, was increased to three times that of the control when exsufflation was applied (Table I).

Respiratory acidosis was produced in eight patients with emphysema by the administration of 100 per cent oxygen by mask. Cases 5 and 6 received oxygen for 60 and 30 minutes, respectively, and were treated with the exsufflator immediately following cessation of oxygen therapy. The exsufflator was applied for 30 minutes in Case 5 and 15 minutes in Case 6. Cases 7 to 12 received oxygen for 90 minutes and exsufflation for the last 30 minutes of administration.

In all eight cases oxygen caused a rise in arterial carbon dioxide tension and a fall in arterial pH. Table II shows that in Cases 5 and 6 exsufflation promptly reversed these changes, the values being shifted towards normal. The reversal of acid-base disturbance when exsufflation was applied in Case 7, despite the continued administration of 100 per cent oxygen is illustrated in Table III.

Determinations of ventilation and blood gases were also made two hours after the experiment in Cases 8 to 12. Figure 2 shows that in three out of five cases the pH remained elevated and the pCO_2 was maintained at a lower level two hours

TABLE I

The effect of the respirator and exsufflator on tidal volume

Case no.	Control ml.	Respirator ml.	Exsufflator ml.
1	360	312	1,133
2	473	467	1,400
3	312	369	1,075
4	588	569	1,700

after the period of exsufflation, but that the oxygen saturation fell to below the control value in four out of five cases.

The usefulness of the exsufflator in the treatment of a patient with severe emphysema who developed a marked respiratory acidosis was demonstrated in the following case:

Case 13 (A. M.)—A 53 year old white man, was admitted to the hospital on July 5, 1952, complaining of severe dyspnea of seven months duration. He had been in good health until January, 1952, when he developed tightness in his chest, dyspnea and cough with expectoration of green sputum. Despite penicillin therapy these symptoms increased in severity and he required admission to hospital for a short time in February and again in March. He was found to have pulmonary emphysema with bronchopneumonia and possibly an early cor pulmonale. Vital capacity was 2.0 liters. He improved markedly when penicillin, aureomycin and digitalis were administered and phlebotomy performed and was discharged from hospital. On June 15, his dyspnea increased and by July 4, 1952, his symptoms had become very severe. He was then given continuous oxygen by mask at 6 to 7 liters per minute. Drowsiness was noted and he was taken to hospital.

On admission he was drowsy, cyanotic and extremely dyspneic. The jugular veins were distended. Chest was barrel-shaped and movement was limited bilaterally. Hyperresonance, distant breath sounds and bilateral scattered ronchi were present. Blood pressure was 140/85. The liver was palpable one and one-half finger breadths

TABLE II

The effect of exsufflation on acid-base disturbance produced by oxygen therapy in pulmonary emphysema

Case no.	State	Minute volume (*L./min.*)	Arterial pH	Arterial pCO_2 (*mm. Hg*)
5	Rest	7.65	7.38	46
	O_2 60 min.	6.90	7.33	52
	Exsuff. 30 min.	9.90	7.42	39
6	Rest	9.46	7.35	64
	O_2 30 min.	6.85	7.30	77
	Exsuff. 15 min.	12.74	7.36	57

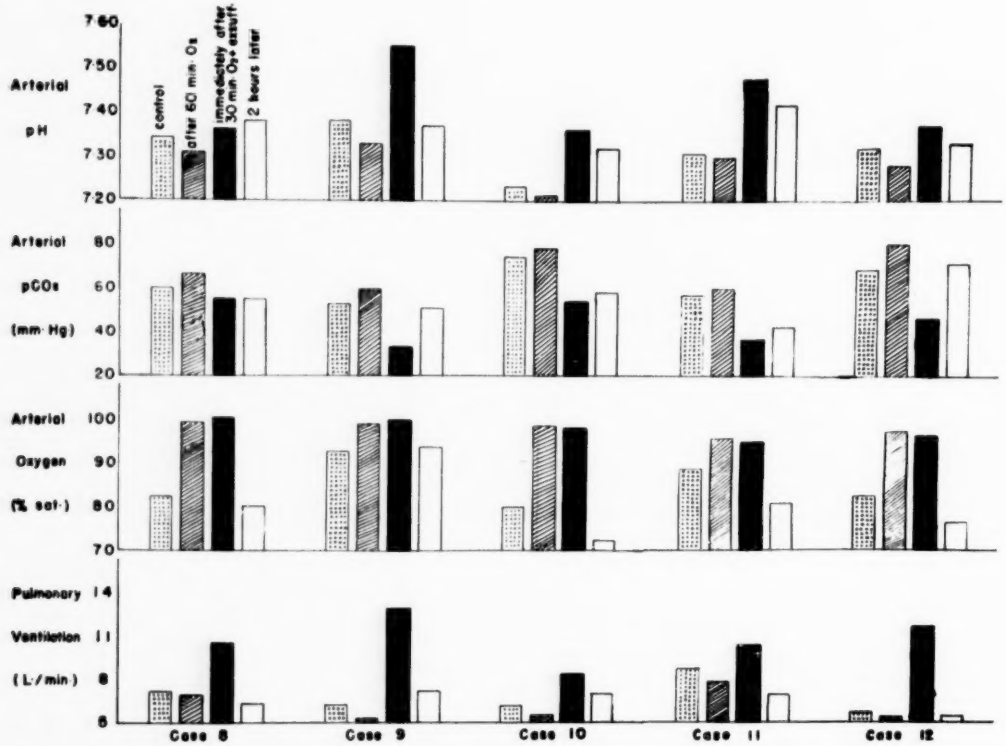

FIG. 2. RESPIRATORY GASES BEFORE AND AFTER EXSUFFLATION IN FIVE PATIENTS WITH EMPHYSEMA MADE ACIDOTIC WITH OXYGEN THERAPY

TABLE III

The effect of exsufflation during continuous oxygen therapy in a patient with emphysema

State	Minute volume (*L./min.*)	Oxygen saturation %	Arterial pH	Arterial pCO_2 (*mm. Hg*)
Resting	6.8	92.8	7.44	44
Oxygen 60 min.	5.9	98.1	7.36	55
Oxygen and exsufflation	9.2	99.0	7.42	43

below the costal margin. There was pitting edema of both ankles and slight clubbing of the fingers. Haemoglobin was 15.5 Gm. per cent; red blood cells, 7.8 million per cmm.; hematocrit, 55 per cent; and white blood cells, 11,600 per cmm. Vital capacity was 1.0 liters. The chest film revealed bullous emphysema with some right ventricular enlargement. EKG showed right axis deviation.

On July 7, further oxygen was administered and he became almost apneic. The arterial pH was 7.31 and the carbon dioxide tension 84 mm. Hg. He was placed in a tank respirator and also received exsufflation for ½ hour every 2 hours. A comparison of the effect of the respirator and exsufflator on tidal volume and minute ventilation is shown in Table IV. He was treated by the combined use of the respirator and exsufflator and oxygen by nasal catheter at 1 to 3 liters per min. until July 10, 1952. The improvements in arterial pH and carbon dioxide tension are shown in Table V. By July 20th he was up and about though limited in activity, and discharged from hospital.

DISCUSSION

The results reported above indicate that the exsufflator is superior to the conventional respirator in managing the acid-base disturbance associated with respiratory acidosis in pulmonary emphysema. This might be explained by the ability of the exsufflator both to overcome obstruction to breathing (9) and to ventilate the alveoli more effectively.

The presence of bronchiolar obstruction due to either spasm or thick viscid secretions in the bronchi is probably a considerable factor in the development of anoxia and carbon dioxide retention in severe emphysema. The exsufflator has been shown to result in a marked elimination of secretions in many cases of emphysema (13). This effect could thus play a large role in the shift towards normal of the arterial blood gases and pH.

TABLE IV

The effect of the respirator and exsufflator on tidal volume and minute ventilation (Case 13)

	Tidal volume (*ml.*)	Minute volume (*L./min.*)
Control	286	5.2
Respirator	278	4.7
Exsufflator	645	5.8

TABLE V

The effect of therapy on arterial blood estimations (Case 13)

Date	Arterial pH	Arterial pCO_2	Comment
7/7/52	7.31	84	Prior to therapy
7/8/52	7.43	67	During therapy
7/9/52	7.39	66	
7/11/52	7.38	63	Day after cessation of therapy
7/15/52	7.40	64	Out of bed, limited activity

In the cases treated in the conventional respirator the tidal volume and minute ventilation did not change appreciably from that present during unassisted respiration. However, a consistently marked increase in tidal volume occurred during the period of exsufflation. The increase in arterial oxygen saturation and fall in carbon dioxide tension despite a respiratory rate of only 9 times a minute is due to the threefold increase in tidal volume resulting in a more effective alveolar ventilation. Case 13 demonstrates that despite only a slight increase in minute ventilation, the marked increase in tidal air produced beneficial results.

No post-exsufflator period of apnea was observed despite the fact that the stimulus of anoxia was removed. It seems possible that the sensitivity of the respiratory mechanisms was at least partially restored by the changes in gas tension induced by the exsufflator. It is also noteworthy that in Cases 8, 10, and 11 the O_2 saturation at two hours was below the original control level while the CO_2 tension and pH were maintained at closer to normal values. This anomalous effect on arterial O_2 and CO_2 levels was presumably due to an increased ventilation of normal alveoli occurring at the expense of ventilation of malfunctioning parts of the lung.

It is concluded that exsufflation is a helpful adjunct in the handling of the problem presented by retention of carbon dioxide in a patient with severe pulmonary emphysema. During exsufflation, oxygen may be administered to such patients, thereby relieving the consequences of severe anoxia without inducing respiratory acidosis. However, as a

sequel to the maintenance of a lowered arterial pCO_2 the arterial blood may become more anoxic following cessation of therapy.

SUMMARY

In four cases of pulmonary emphysema, 30 minutes of therapy with the exsufflator resulted in an increased minute ventilation and beneficial effect on arterial pH, carbon dioxide tension, and oxygen saturation. Respirator therapy for 60 minutes resulted in no appreciable change.

Uncompensated respiratory acidosis was produced in eight cases of emphysema by the administration of oxygen. The acid-base disturbance was effectively treated by a short period of exsufflation. In three of five cases the low level of CO_2 was maintained after two hours while the oxygen saturation fell below the control level. The beneficial effect of the use of the exsufflator in the treatment of a patient with emphysema who developed respiratory acidosis was demonstrated.

The decisive increase in tidal air and minute ventilation with the rapid effect on pulmonary gas exchange during exsufflation appears to be a valuable adjunct to therapy in respiratory acidosis.

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Volume 12, No. 4

Issued November, 1953

CONTENTS

Page

1. **The Functions of the Organs of Zuckerkandl.** By G. B. West, D. M. Shepherd, R. B. Hunter and Agnes R. MacGregor 317

2. **The Plasma Volume in Glomerulo-nephritis.** By A. W. Steinbeck . . 327

3. **The Distribution of Water, Sodium and Potassium in Heart and Skeletal Muscle in Experimental Renal Hypertension in Rats.** By J. M. Ledingham 337

4. **Red Cell Life-span in Nephritis and in Hepatic Cirrhosis.** By H. Chaplin, Jun., and P. L. Mollison 351

5. **The Blood Flow in Human Muscle during Cycloproprane Anaesthesia.** By A. H. Kitchen, C. Sanger, H. E. de Wardener and I. Maureen Young 361

6. **Cantharides Blisters in Children with Rheumatic Fever.** By E. M. Ansell, S. Antonini and L. E. Glynn 367

7. **The Secretion of Radio-active Phosphorus (^{32}P) in Saliva and Gastric Juice in Man.** By J. Stevens 375

8. **The Utilisation of Folic Acid in Anaemia and Leukaemia.** By G. H. Spray and L. J. Witts 387

9. **The Utilisation of Folinic Acid Injected Intravenously.** By G. H. Spray and L. J. Witts 391

10. **Study of Effect of Perchlorate and Thiocyanate on Salivary Iodide.** By E. N. Rowlands, D. A. W. Edwards and A. J. Honour 399

11. **The Circulation in the Chronically Denervated Forearm.** By F. Duff and J. T. Shepherd 407

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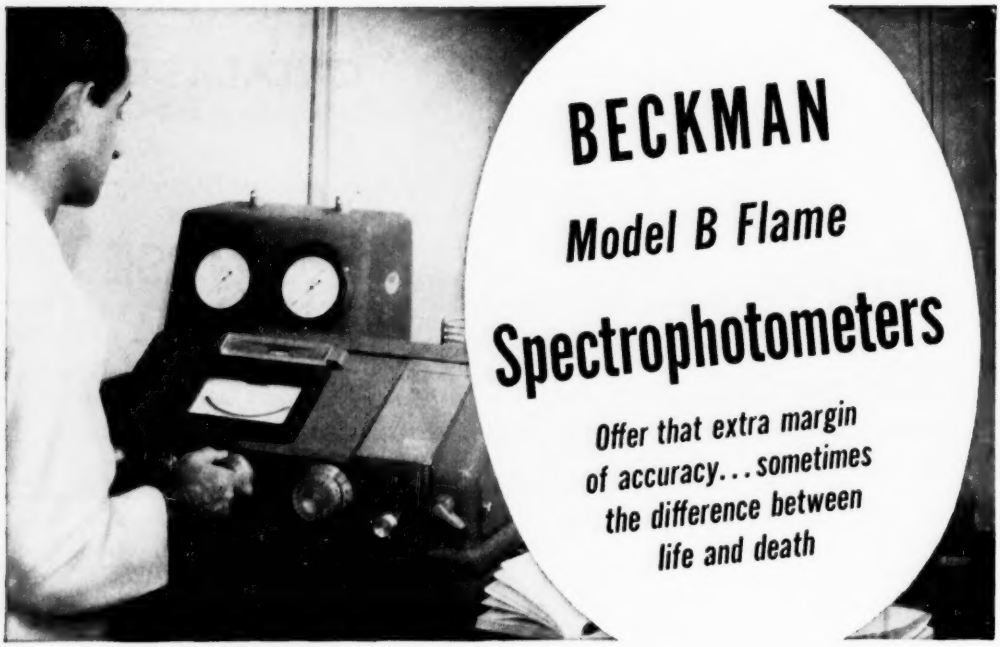
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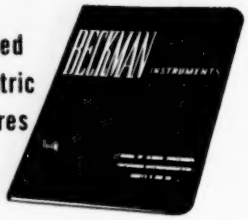

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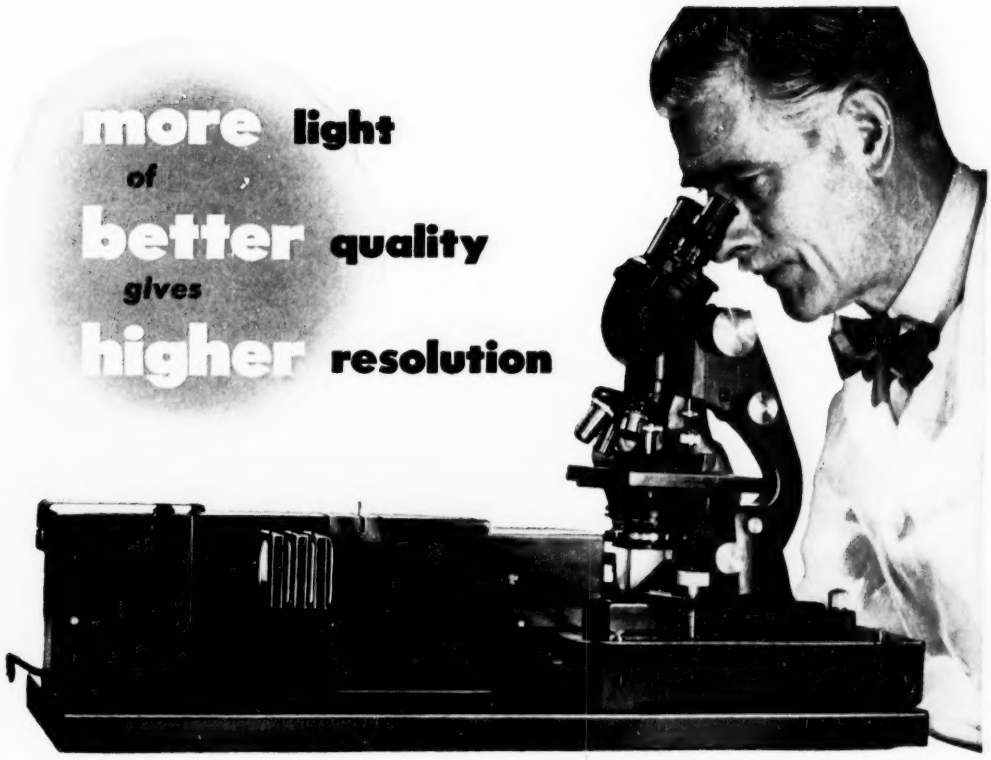

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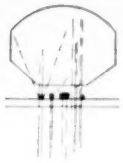

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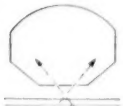

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